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By: ______________________

April 13, 2000

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RE: New Patent Application Transmittal

Sir:

Kindly award a filing date and serial number under 35 USC 111 to the patent application based upon the enclosed specification (and any drawings). Declaration and filing fee are deferred. Please direct all correspondence to the undersigned at the address indicated below.

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TITLE: NUCLEIC ACID SEQUENCES TO PROTEINS INVOLVED IN TOCOPHEROL SYNTHESIS

[X] Specification (55 total pages)

[X] Sheets of Drawings (92 sheets)

[ ] This application claims the benefit of the filing date of US. Application Serial Number *.

[ ] Sequence Listing. Provided herein are a formatted Sequence Listing and a copy of the Sequence Listing on computer readable form as required by 37 CFR 1.821 through 1.825

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Enclosure

NUCLEIC ACID SEQUENCES TO PROTEINS INVOLVED IN TOCOPHEROL SYNTHESIS

INTRODUCTION

TECHNICAL FIELD

The present invention is directed to nucleic acid and amino acid sequences and constructs, and methods related thereto.

BACKGROUND

Isoprenoids are ubiquitous compounds found in all living organisms. Plants synthesize a diverse array of greater than 22,000 isoprenoids (Connolly and Hill (1992) *Dictionary of Terpenoids*, Chapman and Hall, New York, NY). In plants, isoprenoids play essential roles in particular cell functions such as production of sterols, contributing to eukaryotic membrane architecture, acyclic polyprenoids found in the side chain of ubiquinone and plastoquinone, growth regulators like abscisic acid, gibberellins, brassinosteroids or the photosynthetic pigments chlorophylls and carotenoids. Although the physiological role of other plant isoprenoids is less evident, like that of the vast array of secondary metabolites, some are known to play key roles mediating the adaptative responses to different environmental challenges. In spite of the remarkable diversity of structure and function, all isoprenoids originate from a single metabolic precursor, isopentenyl diphosphate (IPP) (Wright, (1961) *Annu. Rev. Biochem.* 20:525-548; and Spurgeon and Porter, (1981) in Biosynthesis of Isoprenoid Compounds., Porter and Spurgeon eds (John Wiley, New York) Vol. 1, pp1-46).

A number of unique and interconnected biochemical pathways derived from the isoprenoid pathway leading to secondary metabolites, including tocopherols, exist in chloroplasts of higher plants. Tocopherols not only perform vital functions in plants, but are also important from mammalian nutritional perspectives. In plastids, tocopherols account for up to 40% of the total quinone pool.

Tocopherols and tocotrienols (unsaturated tocopherol derivatives) are well known antioxidants, and play an important role in protecting cells from free radical damage, and in the prevention of many diseases, including cardiac disease, cancer, cataracts, retinopathy, Alzheimer's disease, and neurodegeneration, and have been shown to have beneficial effects on symptoms of arthritis, and in anti-aging. Vitamin E is used in chicken feed for improving the shelf life, appearance, flavor, and oxidative stability of meat, and to transfer tocols from feed to eggs. Vitamin E has been shown to be essential for normal reproduction, improves overall performance, and enhances immunocompetence in livestock animals. Vitamin E supplement in animal feed also imparts oxidative stability to milk products.

The demand for natural tocopherols as supplements has been steadily growing at a rate of 10-20% for the past three years. At present, the demand exceeds the supply for natural tocopherols, which are known to be more biopotent than racemic mixtures of synthetically produced tocopherols. Naturally occurring tocopherols are all *d*-stereomers, whereas synthetic α-tocopherol is a mixture of eight *d,l*-α-tocopherol isomers, only one of which (12.5%) is identical to the natural *d*-α-tocopherol. Natural *d*-α-tocopherol has the highest vitamin E activity (1.49 IU/mg) when compared to other natural tocopherols or tocotrienols. The synthetic α-tocopherol has a vitamin E activity of 1.1 IU/mg. In 1995, the worldwide market for raw refined tocopherols was $1020 million; synthetic materials comprised 85-88% of the market, the remaining 12-15% being natural materials. The best sources of natural tocopherols and tocotrienols are vegetable oils and grain products. Currently, most of the natural Vitamin E is produced from γ-tocopherol derived from soy oil processing, which is subsequently converted to α-tocopherol by chemical modification (α-tocopherol exhibits the greatest biological activity).

Methods of enhancing the levels of tocopherols and tocotrienols in plants, especially levels of the more desirable compounds that can be used directly, without chemical modification, would be useful to the art as such molecules exhibit better functionality and biovailability.

In addition, methods for the increased production of other isoprenoid derived compounds in a host plant cell is desirable. Furthermore, methods for the production of particular isoprenoid compounds in a host plant cell is also needed.

SUMMARY OF THE INVENTION

The present invention is directed to sequences to proteins involved in tocopherol synthesis. The polynucleotides and polypeptides of the present invention include those derived from prokaryotic and eukaryotic sources.

Thus, one aspect of the present invention relates to prenyltransferase, and in particular to isolated polynucleotide sequences encoding prenyltransferase proteins and polypeptides related thereto. In particular, isolated nucleic acid sequences encoding prenyltransferase proteins from bacterial and plant sources are provided.

In another aspect, the present invention provides isolated polynucleotide sequences encoding tocopherol cyclase, and polypeptides related thereto. In particular, isolated nucleic acid sequences encoding tocopherol cyclase proteins from bacterial and plant sources are provided.

Another aspect of the present invention relates to oligonucleotides which include partial or complete prenyltransferase or tocopherol cyclase encoding sequences.

It is also an aspect of the present invention to provide recombinant DNA constructs which can be used for transcription or transcription and translation (expression) of prenyltransferase or tocopherol cyclase. In particular, constructs are provided which are capable of transcription or transcription and translation in host cells.

In another aspect of the present invention, methods are provided for production of prenyltransferase or tocopherol cyclase in a host cell or progeny thereof. In particular, host cells are transformed or transfected with a DNA construct which can be used for transcription or transcription and translation of prenyltransferase or tocopherol cyclase. The recombinant cells which contain prenyltransferase or tocopherol cyclase are also part of the present invention.

In a further aspect, the present invention relates to methods of using polynucleotide and polypeptide sequences to modify the tocopherol content of host cells, particularly in host plant cells. Plant cells having such a modified tocopherol content are also contemplated herein. Methods and cells in which both prenyltransferase and tocopherol cyclase are expressed in a host cell are also part of the present invention.

The modified plants, seeds and oils obtained by the expression of the prenyltransferase or tocopherol cyclase are also considered part of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides an amino acid sequence alignment between ATPT2, ATPT3, ATPT4, ATPT8, and ATPT12 are performed using ClustalW.

Figure 2 provides a schematic picture of the expression construct pCGN10800.

Figure 3 provides a schematic picture of the expression construct pCGN10801.

Figure 4 provides a schematic picture of the expression construct pCGN10803.

Figure 5 provides a schematic picture of the construct pCGN10806.

Figure 6 provides a schematic picture of the construct pCGN10807.

Figure 7 provides a schematic picture of the construct pCGN10808.

Figure 8 provides a schematic picture of the expression construct pCGN10809.

Figure 9 provides a schematic picture of the expression construct pCGN10810.

Figure 10 provides a schematic picture of the expression construct pCGN10811.

Figure 11 provides a schematic picture of the expression construct pCGN10812.

Figure 12 provides a schematic picture of the expression construct pCGN10813.

Figure 13 provides a schematic picture of the expression construct pCGN10814.

Figure 14 provides a schematic picture of the expression construct pCGN10815.

Figure 15 provides a schematic picture of the expression construct pCGN10816.

Figure 16 provides a schematic picture of the expression construct pCGN10817.

Figure 17 provides a schematic picture of the expression construct pCGN10819.

Figure 18 provides a schematic picture of the expression construct pCGN10824.

Figure 19 provides a schematic picture of the expression construct pCGN10825.

Figure 20 provides a schematic picture of the expression construct pCGN10826.

Figure 21 provides an amino acid sequence alignment using ClustalW between the *Synechocystis* prenyltransferase sequences.

Figure 22 provides an amino acid sequence of the ATPT2, ATPT3, ATPT4, ATPT8, and ATPT12 protein sequences from *Arabidopsis* and the slr1736, slr0926, sll1899, slr0056, and the slr1518 amino acid sequences from *Synechocystis*.

Figure 23 provides the results of the enzymatic assay from preparations of wild type *Synechocystis* strain 6803, and *Synechocystis* slr1736 knockout.

Figure 24 provides bar graphs of HPLC data obtained from seed extracts of transgenic *Arabidopsis* containing pCGN10822, which provides of the expression of the ATPT2 sequence, in the sense orientation, from the napin promoter. Provided are graphs for alpha, gamma, and delta tocopherols, as well as total tocopherol for 22 transformed lines, as well as a nontransformed (wildtype) control.

Figure 25 provides a bar graph of HPLC analysis of seed extracts from *Arabidopsis* plants transformed with pCGN10803 (35S-ATPT2, in the antisense orientation), pCGN10822 (line 1625, napin ATPT2 in the sense orientation), pCGN10809 (line 1627, 35S-ATPT3 in the sense orientation), a nontransformed (wt) control, and an empty vector transformed control.

Figure 26 shows total tocopherol levels measured in T# *Arabidopsis* seed of line.

Figure 27 shows total tocopherol levels measured in T# *Arabidopsis* seed of line.

Figure 28 shows total tocopherol levels measured in developing canola seed of line 10822-1.

Figure 29: shows results of phytyl prenyltransferase activity assay using *Synechocystis* wild type and slr1737 knockout mutant membrane preparations.

Figure 30 is the chromatograph from an HPLC analysis of *Synechocystis* extracts.

Figure 31 is a sequence alignment of the *Arabidopsis* homologue with the sequence of the public database.

Figure 32 shows the results of hydropathic analysis of slr1737

Figure 33 shows the results of hydropathic analysis of the *Arabidopsis* homologue of slr1737.

Figure 34 shows the catalytic mechanism of various cyclase enzymes

Figure 35 is a sequence alignment of slr1737, slr1737 *Arabidopsis* homologue and the *Arabidopsis* chalcone isomerase.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides, *inter alia*, compositions and methods for altering (for example, increasing and decreasing) the tocopherol levels and/or modulating their ratios in host cells. In particular, the present invention provides polynucleotides, polypeptides, and methods of use thereof for the modulation of tocopherol content in host plant cells.

The biosynthesis of α-tocopherol in higher plants involves condensation of homogentisic acid and phytylpyrophosphate to form 2-methyl-6 phytylbenzoquinol that can, by cyclization and subsequent methylations (Fiedler et al., 1982, *Planta*, 155: 511-515, Soll et al., 1980, *Arch. Biochem. Biophys*. 204: 544-550, Marshall et al., 1985 *Phytochem*., 24: 1705-1711, all of which are herein incorporated by reference in their entirety), form various tocopherols.

The *Arabidopsis pds2* mutant identified and characterized by Norris *et al.* (1995), is deficient in tocopherol and plastiquinone-9 accumulation. Further genetic and biochemical analysis suggested that the protein encoded by *PDS2* may be responsible for the prenylation of homogentisic acid. The *PDS2* locus identified by Norris *et al.* (1995) has been hypothesized to possibly encode the tocopherol phytyl-prenyltransferase, as the *pds2* mutant fails to accumulate tocopherols.

Norris *et al.* (1995) determined that in *Arabidopsis pds2* lies at the top of chromosome 3, approximately 7 centimorgans above long hypocotyl2, based on the genetic map. ATPT2 is located on chromosome 2 between 36 and 41 centimorgans, lying on BAC F19F24, indicating that ATPT2 does not correspond to *PDS2*. Thus, it is an aspect of the present invention to provide novel polynucleotides and polypeptides involved in the prenylation of homogentisic acid. This reaction may be a rate limiting step in tocopherol biosynthesis, and this gene has yet to be isolated.

U.S. Patent No. 5,432,069 describes the partial purification and characterization of tocopherol cyclase from *Chlorella protothecoides, Dunaliella salina* and wheat. The cyclase described as being glycine rich, water soluble and with a predicted MW of 48-50kDa. However, only limited peptide fragment sequences were available.

In one aspect, the present invention provides polynucleotide and polypeptide sequences involved in the prenylation of straight chain and aromatic compounds. Straight chain prenyltransferases as used herein comprises sequences which encode proteins involved in the prenylation of straight chain compounds, including, but not limited to, geranyl geranyl

pyrophosphate and farnesyl pyrophosphate. Aromatic prenyltransferases, as used herein, comprises sequences which encode proteins involved in the prenylation of aromatic compounds, including, but not limited to, menaquinone, ubiquinone, chlorophyll, and homogentisic acid. The prenyltransferase of the present invention preferably prenylates homogentisic acid.

In another aspect, the invention provides polynucleotide and polypeptide sequences to tocopherol cyclization enzymes. The 2,3-dimethyl-5-phytylplastoquinol cyclase (tocopherol cyclase) is responsible for the cyclization of 2,3-dimethyl-5-phytylplastoquinol to tocopherol.

Isolated Polynucleotides, Proteins, and Polypeptides

A first aspect of the present invention relates to isolated prenyltransferase polynucleotides. Another aspect of the present invention relates to isolated tocopherol cyclase polynucleotides. The polynucleotide sequences of the present invention include isolated polynucleotides that encode the polypeptides of the invention having a deduced amino acid sequence selected from the group of sequences set forth in the Sequence Listing and to other polynucleotide sequences closely related to such sequences and variants thereof.

The invention provides a polynucleotide sequence identical over its entire length to each coding sequence as set forth in the Sequence Listing. The invention also provides the coding sequence for the mature polypeptide or a fragment thereof, as well as the coding sequence for the mature polypeptide or a fragment thereof in a reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, pro-, or prepro- protein sequence. The polynucleotide can also include non-coding sequences, including for example, but not limited to, non-coding 5' and 3' sequences, such as the transcribed, untranslated sequences, termination signals, ribosome binding sites, sequences that stabilize mRNA, introns, polyadenylation signals, and additional coding sequence that encodes additional amino acids. For example, a marker sequence can be included to facilitate the purification of the fused polypeptide. Polynucleotides of the present invention also include polynucleotides comprising a structural gene and the naturally associated sequences that control gene expression.

The invention also includes polynucleotides of the formula:

$$X\text{-}(R_1)_n\text{-}(R_2)\text{-}(R_3)_n\text{-}Y$$

wherein, at the 5' end, X is hydrogen, and at the 3' end, Y is hydrogen or a metal, R_1 and R_3 are any nucleic acid residue, n is an integer between 1 and 3000, preferably between 1 and 1000 and R_2 is a nucleic acid sequence of the invention, particularly a nucleic acid sequence selected from the group set forth in the Sequence Listing and preferably those of SEQ ID NOs: 1, 3, 5, 7, 8, 10, 11, 13-16, 18, 23, 29, 36, and 38. In the formula, R_2 is oriented so that its 5' end residue is at the left, bound to R_1, and its 3' end residue is at the right, bound to R_3. Any stretch of nucleic acid residues denoted by either R group, where R is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer.

The invention also relates to variants of the polynucleotides described herein that encode for variants of the polypeptides of the invention. Variants that are fragments of the polynucleotides of the invention can be used to synthesize full-length polynucleotides of the invention. Preferred embodiments are polynucleotides encoding polypeptide variants wherein 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues of a polypeptide sequence of the invention are substituted, added or deleted, in any combination. Particularly preferred are substitutions, additions, and deletions that are silent such that they do not alter the properties or activities of the polynucleotide or polypeptide.

Further preferred embodiments of the invention that are at least 50%, 60%, or 70% identical over their entire length to a polynucleotide encoding a polypeptide of the invention, and polynucleotides that are complementary to such polynucleotides. More preferable are polynucleotides that comprise a region that is at least 80% identical over its entire length to a polynucleotide encoding a polypeptide of the invention and polynucleotides that are complementary thereto. In this regard, polynucleotides at least 90% identical over their entire length are particularly preferred, those at least 95% identical are especially preferred. Further, those with at least 97% identity are highly preferred and those with at least 98% and 99% identity are particularly highly preferred, with those at least 99% being the most highly preferred.

Preferred embodiments are polynucleotides that encode polypeptides that retain substantially the same biological function or activity as the mature polypeptides encoded by the polynucleotides set forth in the Sequence Listing.

The invention further relates to polynucleotides that hybridize to the above-described sequences. In particular, the invention relates to polynucleotides that hybridize under stringent

conditions to the above-described polynucleotides. As used herein, the terms "stringent conditions" and "stringent hybridization conditions" mean that hybridization will generally occur if there is at least 95% and preferably at least 97% identity between the sequences. An example of stringent hybridization conditions is overnight incubation at 42°C in a solution comprising 50% formamide, 5x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/milliliter denatured, sheared salmon sperm DNA, followed by washing the hybridization support in 0.1x SSC at approximately 65°C. Other hybridization and wash conditions are well known and are exemplified in Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition, cold Spring Harbor, NY (1989), particularly Chapter 11.

The invention also provides a polynucleotide consisting essentially of a polynucleotide sequence obtainable by screening an appropriate library containing the complete gene for a polynucleotide sequence set for in the Sequence Listing under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers as described herein.

As discussed herein regarding polynucleotide assays of the invention, for example, polynucleotides of the invention can be used as a hybridization probe for RNA, cDNA, or genomic DNA to isolate full length cDNAs or genomic clones encoding a polypeptide and to isolate cDNA or genomic clones of other genes that have a high sequence similarity to a polynucleotide set forth in the Sequence Listing. Such probes will generally comprise at least 15 bases. Preferably such probes will have at least 30 bases and can have at least 50 bases. Particularly preferred probes will have between 30 bases and 50 bases, inclusive.

The coding region of each gene that comprises or is comprised by a polynucleotide sequence set forth in the Sequence Listing may be isolated by screening using a DNA sequence provided in the Sequence Listing to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to identify members of the library which hybridize to the probe. For example, synthetic oligonucleotides are prepared which correspond to the prenyltransferase or tocopherol cyclase EST sequences. The oligonucleotides are used as

primers in polymerase chain reaction (PCR) techniques to obtain 5' and 3' terminal sequence of prenyltransferase or tocopherol cyclase genes. Alternatively, where oligonucleotides of low degeneracy can be prepared from particular prenyltransferase or tocopherol cyclase peptides, such probes may be used directly to screen gene libraries for prenyltransferase or tocopherol cyclase gene sequences. In particular, screening of cDNA libraries in phage vectors is useful in such methods due to lower levels of background hybridization.

Typically, a prenyltransferase or tocopherol cyclase sequence obtainable from the use of nucleic acid probes will show 60-70% sequence identity between the target prenyltransferase or tocopherol cyclase sequence and the encoding sequence used as a probe. However, lengthy sequences with as little as 50-60% sequence identity may also be obtained. The nucleic acid probes may be a lengthy fragment of the nucleic acid sequence, or may also be a shorter, oligonucleotide probe. When longer nucleic acid fragments are employed as probes (greater than about 100 bp), one may screen at lower stringencies in order to obtain sequences from the target sample which have 20-50% deviation (i.e., 50-80% sequence homology) from the sequences used as probe. Oligonucleotide probes can be considerably shorter than the entire nucleic acid sequence encoding an prenyltransferase or tocopherol cyclase enzyme, but should be at least about 10, preferably at least about 15, and more preferably at least about 20 nucleotides. A higher degree of sequence identity is desired when shorter regions are used as opposed to longer regions. It may thus be desirable to identify regions of highly conserved amino acid sequence to design oligonucleotide probes for detecting and recovering other related prenyltransferase or tocopherol cyclase genes. Shorter probes are often particularly useful for polymerase chain reactions (PCR), especially when highly conserved sequences can be identified. (*See*, Gould, *et al.*, *PNAS USA* (1989) *86*:1934-1938.).

Another aspect of the present invention relates to prenyltransferase or tocopherol cyclase polypeptides. Such polypeptides include isolated polypeptides set forth in the Sequence Listing, as well as polypeptides and fragments thereof, particularly those polypeptides which exhibit prenyltransferase or tocopherol cyclase activity and also those polypeptides which have at least 50%, 60% or 70% identity, preferably at least 80% identity, more preferably at least 90% identity, and most preferably at least 95% identity to a polypeptide sequence selected from the group of sequences set forth in the Sequence Listing, and also include portions of such

polypeptides, wherein such portion of the polypeptide preferably includes at least 30 amino acids and more preferably includes at least 50 amino acids.

"Identity", as is well understood in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods including, but not limited to, those described in *Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York (1988); *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data, Part I*, Griffin, A.M. and Griffin, H.G., eds., Humana Press, New Jersey (1994); *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press (1987); *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., Stockton Press, New York (1991); and Carillo, H., and Lipman, D., SIAM *J Applied Math*, 48:1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available programs. Computer programs which can be used to determine identity between two sequences include, but are not limited to, GCG (Devereux, J., et al., *Nucleic Acids Research* 12(1):387 (1984); suite of five BLAST programs, three designed for nucleotide sequences queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN) (Coulson, *Trends in Biotechnology, 12:* 76-80 (1994); Birren, *et al., Genome Analysis, 1:* 543-559 (1997)). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., *et al.*, NCBI NLM NIH, Bethesda, MD 20894; Altschul, S., *et al.*, *J. Mol. Biol.*, 215:403-410 (1990)). The well known Smith Waterman algorithm can also be used to determine identity.

Parameters for polypeptide sequence comparison typically include the following:

Algorithm: Needleman and Wunsch, *J. Mol. Biol.* 48:443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci USA* 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

A program which can be used with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison Wisconsin. The above parameters along with no penalty for end gap are the default parameters for peptide comparisons.

Parameters for polynucleotide sequence comparison include the following:

Algorithm: Needleman and Wunsch, J. Mol. Biol. 48:443-453 (1970)

Comparison matrix: matches = +10; mismatches = 0

Gap Penalty: 50

Gap Length Penalty: 3

A program which can be used with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison Wisconsin. The above parameters are the default parameters for nucleic acid comparisons.

The invention also includes polypeptides of the formula:

$$X\text{-}(R_1)_n\text{-}(R_2)\text{-}(R_3)_n\text{-}Y$$

wherein, at the amino terminus, X is hydrogen, and at the carboxyl terminus, Y is hydrogen or a metal, R_1 and R_3 are any amino acid residue, n is an integer between 1 and 1000, and R_2 is an amino acid sequence of the invention, particularly an amino acid sequence selected from the group set forth in the Sequence Listing and preferably those encoded by the sequences provided in SEQ ID NOs: 2, 4, 6, 9, 12, 17, 19-22, 24-28, 30, 32-35, 37, and 39. In the formula, R_2 is oriented so that its amino terminal residue is at the left, bound to R_1, and its carboxy terminal residue is at the right, bound to R_3. Any stretch of amino acid residues denoted by either R group, where R is greater than 1, may be either a heteropolymer or a homopolymer, preferably a heteropolymer.

Polypeptides of the present invention include isolated polypeptides encoded by a polynucleotide comprising a sequence selected from the group of a sequence contained in the Sequence Listing set forth herein .

The polypeptides of the present invention can be mature protein or can be part of a fusion protein.

Fragments and variants of the polypeptides are also considered to be a part of the invention. A fragment is a variant polypeptide which has an amino acid sequence that is entirely the same as part but not all of the amino acid sequence of the previously described polypeptides.

The fragments can be "free-standing" or comprised within a larger polypeptide of which the fragment forms a part or a region, most preferably as a single continuous region. Preferred fragments are biologically active fragments which are those fragments that mediate activities of the polypeptides of the invention, including those with similar activity or improved activity or with a decreased activity. Also included are those fragments that antigenic or immunogenic in an animal, particularly a human.

Variants of the polypeptide also include polypeptides that vary from the sequences set forth in the Sequence Listing by conservative amino acid substitutions, substitution of a residue by another with like characteristics. In general, such substitutions are among Ala, Val, Leu and Ile; between Ser and Thr; between Asp and Glu; between Asn and Gln; between Lys and Arg; or between Phe and Tyr. Particularly preferred are variants in which 5 to 10; 1 to 5; 1 to 3 or one amino acid(s) are substituted, deleted, or added, in any combination.

Variants that are fragments of the polypeptides of the invention can be used to produce the corresponding full length polypeptide by peptide synthesis. Therefore, these variants can be used as intermediates for producing the full-length polypeptides of the invention.

The polynucleotides and polypeptides of the invention can be used, for example, in the transformation of host cells, such as plant host cells, as further discussed herein.

The invention also provides polynucleotides that encode a polypeptide that is a mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids within the mature polypeptide (for example, when the mature form of the protein has more than one polypeptide chain). Such sequences can, for example, play a role in the processing of a protein from a precursor to a mature form, allow protein transport, shorten or lengthen protein half-life, or facilitate manipulation of the protein in assays or production. It is contemplated that cellular enzymes can be used to remove any additional amino acids from the mature protein.

A precursor protein, having the mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. The inactive precursors generally are activated when the prosequences are removed. Some or all of the prosequences may be removed prior to activation. Such precursor protein are generally called proproteins.

Plant Constructs and Methods of Use

Of particular interest is the use of the nucleotide sequences in recombinant DNA constructs to direct the transcription or transcription and translation (expression) of the prenyltransferase or tocopherol cyclase sequences of the present invention in a host plant cell. The expression constructs generally comprise a promoter functional in a host plant cell operably linked to a nucleic acid sequence encoding a prenyltransferase or tocopherol cyclase of the present invention and a transcriptional termination region functional in a host plant cell.

A first nucleic acid sequence is "operably linked" or "operably associated" with a second nucleic acid sequence when the sequences are so arranged that the first nucleic acid sequence affects the function of the second nucleic-acid sequence. Preferably, the two sequences are part of a single contiguous nucleic acid molecule and more preferably are adjacent. For example, a promoter is operably linked to a gene if the promoter regulates or mediates transcription of the gene in a cell.

Those skilled in the art will recognize that there are a number of promoters which are functional in plant cells, and have been described in the literature. Chloroplast and plastid specific promoters, chloroplast or plastid functional promoters, and chloroplast or plastid operable promoters are also envisioned.

One set of plant functional promoters are constitutive promoters such as the CaMV35S or FMV35S promoters that yield high levels of expression in most plant organs. Enhanced or duplicated versions of the CaMV35S and FMV35S promoters are useful in the practice of this invention (Odell, *et al.* (1985) *Nature* 313:810-812; Rogers, U.S. Patent Number 5,378, 619). In addition, it may also be preferred to bring about expression of the prenyltransferase or tocopherol cyclase gene in specific tissues of the plant, such as leaf, stem, root, tuber, seed, fruit, etc., and the promoter chosen should have the desired tissue and developmental specificity.

Of particular interest is the expression of the nucleic acid sequences of the present invention from transcription initiation regions which are preferentially expressed in a plant seed tissue. Examples of such seed preferential transcription initiation sequences include those sequences derived from sequences encoding plant storage protein genes or from genes involved in fatty acid biosynthesis in oilseeds. Examples of such promoters include the 5' regulatory regions from such genes as napin (Kridl *et al., Seed Sci. Res. 1:*209:219 (1991)), phaseolin, zein,

soybean trypsin inhibitor, ACP, stearoyl-ACP desaturase, soybean α' subunit of β-conglycinin (soy 7s, (Chen *et al.*, *Proc. Natl. Acad. Sci.*, 83:8560-8564 (1986))) and oleosin.

It may be advantageous to direct the localization of proteins conferring prenyltransferase or tocopherol cyclase to a particular subcellular compartment, for example, to the mitochondrion, endoplasmic reticulum, vacuoles, chloroplast or other plastidic compartment. For example, where the genes of interest of the present invention will be targeted to plastids, such as chloroplasts, for expression, the constructs will also employ the use of sequences to direct the gene to the plastid. Such sequences are referred to herein as chloroplast transit peptides (CTP) or plastid transit peptides (PTP). In this manner, where the gene of interest is not directly inserted into the plastid, the expression construct will additionally contain a gene encoding a transit peptide to direct the gene of interest to the plastid. The chloroplast transit peptides may be derived from the gene of interest, or may be derived from a heterologous sequence having a CTP. Such transit peptides are known in the art. See, for example, Von Heijne *et al.* (1991) *Plant Mol. Biol. Rep. 9*:104-126; Clark *et al.* (1989) *J. Biol. Chem. 264*:17544-17550; della-Cioppa *et al.* (1987) *Plant Physiol. 84*:965-968; Romer *et al.* (1993) *Biochem. Biophys. Res Commun. 196*:1414-1421; and, Shah *et al.* (1986) *Science 233*:478-481.

Depending upon the intended use, the constructs may contain the nucleic acid sequence which encodes the entire prenyltransferase or tocopherol cyclase protein, or a portion thereof. For example, where antisense inhibition of a given prenyltransferase or tocopherol cyclase protein is desired, the entire prenyltransferase or tocopherol cyclase sequence is not required. Furthermore, where prenyltransferase or tocopherol cyclase sequences used in constructs are intended for use as probes, it may be advantageous to prepare constructs containing only a particular portion of a prenyltransferase or tocopherol cyclase encoding sequence, for example a sequence which is discovered to encode a highly conserved prenyltransferase or tocopherol cyclase region.

The skilled artisan will recognize that there are various methods for the inhibition of expression of endogenous sequences in a host cell. Such methods include, but are not limited to, antisense suppression (Smith, *et al.* (1988) *Nature* 334:724-726) , co-suppression (Napoli, *et al.* (1989) *Plant Cell* 2:279-289), ribozymes (PCT Publication WO 97/10328), and combinations of sense and antisense Waterhouse, *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95:13959-13964.

Methods for the suppression of endogenous sequences in a host cell typically employ the transcription or transcription and translation of at least a portion of the sequence to be suppressed. Such sequences may be homologous to coding as well as non-coding regions of the endogenous sequence.

Regulatory transcript termination regions may be provided in plant expression constructs of this invention as well. Transcript termination regions may be provided by the DNA sequence encoding the prenyltransferase or tocopherol cyclase or a convenient transcription termination region derived from a different gene source, for example, the transcript termination region which is naturally associated with the transcript initiation region. The skilled artisan will recognize that any convenient transcript termination region which is capable of terminating transcription in a plant cell may be employed in the constructs of the present invention.

Alternatively, constructs may be prepared to direct the expression of the prenyltransferase or tocopherol cyclase sequences directly from the host plant cell plastid. Such constructs and methods are known in the art and are generally described, for example, in Svab, *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:8526-8530 and Svab and Maliga (1993) *Proc. Natl. Acad. Sci. USA* 90:913-917 and in U.S. Patent Number 5,693,507.

The prenyltransferase or tocopherol cyclase constructs of the present invention can be used in transformation methods with additional constructs providing for the expression of other nucleic acid sequences encoding proteins involved in the production of tocopherols, or tocopherol precursors such as homogentisic acid and/or phytylpyrophosphate. Nucleic acid sequences encoding proteins involved in the production of homogentisic acid are known in the art, and include but not are limited to, 4-hydroxyphenylpyruvate dioxygenase (HPPD, EC 1.13.11.27) described for example, by Garcia, *et al.* ((1999) *Plant Physiol.* 119(4):1507-1516), mono or bifunctional tyrA (described for example by Xia, *et al.* (1992) *J. Gen Microbiol.* 138:1309-1316, and Hudson, *et al.* (1984) *J. Mol. Biol.* 180:1023-1051), Oxygenase, 4-hydroxyphenylpyruvate di- (9CI), 4-Hydroxyphenylpyruvate dioxygenase; p-Hydroxyphenylpyruvate dioxygenase; p-Hydroxyphenylpyruvate hydroxylase; p-Hydroxyphenylpyruvate oxidase; p-Hydroxyphenylpyruvic acid hydroxylase; p-Hydroxyphenylpyruvic hydroxylase; p-Hydroxyphenylpyruvic oxidase), 4-hydroxyphenylacetate, NAD(P)H:oxygen oxidoreductase (1-hydroxylating); 4-hydroxyphenylacetate 1-monooxygenase,

and the like. In addition, constructs for the expression of nucleic acid sequences encoding proteins involved in the production of phytylpyrophosphate can also be employed with the prenyltransferase or tocopherol cyclase constructs of the present invention. Nucleic acid sequences encoding proteins involved in the production of phytylpyrophosphate are known in the art, and include, but are not limited to geranylgeranylpyrophosphate synthase (GGPPS), geranylgeranylpyrophosphate reductase (GGH), 1-deoxyxylulose-5-phosphate synthase, 1-deoxy-D-xylolose-5-phosphate reductoisomerase, 4-diphosphocytidyl-2-C-methylerythritol synthase, isopentyl pyrophosphate isomerase.

The prenyltransferase or tocopherol cyclase sequences of the present invention find use in the preparation of transformation constructs having a second expression cassette for the expression of additional sequences involved in tocopherol biosynthesis. Additional tocopherol biosynthesis sequences of interest in the present invention include, but are not limited to gamma-tocpherol methyltransferase (Shintani, *et al.* (1998) *Science* 282(5396):2098-2100), tocopherol cyclase, and tocopherol methyltransferase.

A plant cell, tissue, organ, or plant into which the recombinant DNA constructs containing the expression constructs have been introduced is considered transformed, transfected, or transgenic. A transgenic or transformed cell or plant also includes progeny of the cell or plant and progeny produced from a breeding program employing such a transgenic plant as a parent in a cross and exhibiting an altered phenotype resulting from the presence of a prenyltransferase or tocopherol cyclase nucleic acid sequence.

Plant expression or transcription constructs having a prenyltransferase or tocopherol cyclase as the DNA sequence of interest for increased or decreased expression thereof may be employed with a wide variety of plant life, particularly, plant life involved in the production of vegetable oils for edible and industrial uses. Particularly preferred plants for use in the methods of the present invention include, but are not limited to: *Acacia,* alfalfa, aneth, apple, apricot, artichoke, arugula, asparagus, avocado, banana, barley, beans, beet, blackberry, blueberry, broccoli, brussels sprouts, cabbage, canola, cantaloupe, carrot, cassava, cauliflower, celery, cherry, chicory, cilantro, citrus, clementines, coffee, corn, cotton, cucumber, Douglas fir, eggplant, endive, escarole, eucalyptus, fennel, figs, garlic, gourd, grape, grapefruit, honey dew, jicama, kiwifruit, lettuce, leeks, lemon, lime, Loblolly pine, mango, melon, mushroom, nectarine,

nut, oat, oil palm, oil seed rape, okra, onion, orange, an ornamental plant, papaya, parsley, pea, peach, peanut, pear, pepper, persimmon, pine, pineapple, plantain, plum, pomegranate, poplar, potato, pumpkin, quince, radiata pine, radicchio, radish, raspberry, rice, rye, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugarbeet, sugarcane, sunflower, sweet potato, sweetgum, tangerine, tea, tobacco, tomato, triticale, turf, turnip, a vine, watermelon, wheat, yams, and zucchini.

Most especially preferred are temperate oilseed crops. Temperate oilseed crops of interest include, but are not limited to, rapeseed (Canola and High Erucic Acid varieties), sunflower, safflower, cotton, soybean, peanut, coconut and oil palms, and corn. Depending on the method for introducing the recombinant constructs into the host cell, other DNA sequences may be required. Importantly, this invention is applicable to dicotyledyons and monocotyledons species alike and will be readily applicable to new and/or improved transformation and regulation techniques.

Of particular interest, is the use of prenyltransferase or tocopherol cyclase constructs in plants to produce plants or plant parts, including, but not limited to leaves, stems, roots, reproductive, and seed, with a modified content of tocopherols in plant parts having transformed plant cells.

For immunological screening, antibodies to the protein can be prepared by injecting rabbits or mice with the purified protein or portion thereof, such methods of preparing antibodies being well known to those in the art. Either monoclonal or polyclonal antibodies can be produced, although typically polyclonal antibodies are more useful for gene isolation. Western analysis may be conducted to determine that a related protein is present in a crude extract of the desired plant species, as determined by cross-reaction with the antibodies to the encoded proteins. When cross-reactivity is observed, genes encoding the related proteins are isolated by screening expression libraries representing the desired plant species. Expression libraries can be constructed in a variety of commercially available vectors, including lambda gt11, as described in Sambrook, *et al.* (*Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

To confirm the activity and specificity of the proteins encoded by the identified nucleic acid sequences as prenyltransferase or tocopherol cyclase enzymes, *in vitro* assays are performed

in insect cell cultures using baculovirus expression systems. Such baculovirus expression systems are known in the art and are described by Lee, *et al.* U.S. Patent Number 5,348,886, the entirety of which is herein incorporated by reference.

In addition, other expression constructs may be prepared to assay for protein activity utilizing different expression systems. Such expression constructs are transformed into yeast or prokaryotic host and assayed for prenyltransferase or tocopherol cyclase activity. Such expression systems are known in the art and are readily available through commercial sources.

In addition to the sequences described in the present invention, DNA coding sequences useful in the present invention can be derived from algae, fungi, bacteria, mammalian sources, plants, etc. Homology searches in existing databases using signature sequences corresponding to conserved nucleotide and amino acid sequences of prenyltransferase or tocopherol cyclase can be employed to isolate equivalent, related genes from other sources such as plants and microorganisms. Searches in EST databases can also be employed. Furthermore, the use of DNA sequences encoding enzymes functionally enzymatically equivalent to those disclosed herein, wherein such DNA sequences are degenerate equivalents of the nucleic acid sequences disclosed herein in accordance with the degeneracy of the genetic code, is also encompassed by the present invention. Demonstration of the functionality of coding sequences identified by any of these methods can be carried out by complementation of mutants of appropriate organisms, such as *Synechocystis, Shewanella, yeast, Pseudomonas, Rhodobacteria*, etc., that lack specific biochemical reactions, or that have been mutated. The sequences of the DNA coding regions can be optimized by gene resynthesis, based on codon usage, for maximum expression in particular hosts.

For the alteration of tocopherol production in a host cell, a second expression construct can be used in accordance with the present invention. For example, the prenyltransferase or tocopherol cyclase expression construct can be introduced into a host cell in conjunction with a second expression construct having a nucleotide sequence for a protein involved in tocopherol biosynthesis.

The method of transformation in obtaining such transgenic plants is not critical to the instant invention, and various methods of plant transformation are currently available. Furthermore, as newer methods become available to transform crops, they may also be directly

applied hereunder. For example, many plant species naturally susceptible to *Agrobacterium* infection may be successfully transformed via tripartite or binary vector methods of *Agrobacterium* mediated transformation. In many instances, it will be desirable to have the construct bordered on one or both sides by T-DNA, particularly having the left and right borders, more particularly the right border. This is particularly useful when the construct uses *A. tumefaciens* or *A. rhizogenes* as a mode for transformation, although the T-DNA borders may find use with other modes of transformation. In addition, techniques of microinjection, DNA particle bombardment, and electroporation have been developed which allow for the transformation of various monocot and dicot plant species.

Normally, included with the DNA construct will be a structural gene having the necessary regulatory regions for expression in a host and providing for selection of transformant cells. The gene may provide for resistance to a cytotoxic agent, e.g. antibiotic, heavy metal, toxin, etc., complementation providing prototrophy to an auxotrophic host, viral immunity or the like. Depending upon the number of different host species the expression construct or components thereof are introduced, one or more markers may be employed, where different conditions for selection are used for the different hosts.

Where *Agrobacterium* is used for plant cell transformation, a vector may be used which may be introduced into the *Agrobacterium* host for homologous recombination with T-DNA or the Ti- or Ri-plasmid present in the *Agrobacterium* host. The Ti- or Ri-plasmid containing the T-DNA for recombination may be armed (capable of causing gall formation) or disarmed (incapable of causing gall formation), the latter being permissible, so long as the *vir* genes are present in the transformed *Agrobacterium* host. The armed plasmid can give a mixture of normal plant cells and gall.

In some instances where *Agrobacterium* is used as the vehicle for transforming host plant cells, the expression or transcription construct bordered by the T-DNA border region(s) will be inserted into a broad host range vector capable of replication in *E. coli* and *Agrobacterium*, there being broad host range vectors described in the literature. Commonly used is pRK2 or derivatives thereof. See, for example, Ditta, *et al.*, (*Proc. Nat. Acad. Sci., U.S.A.* (1980) 77:7347-7351) and EPA 0 120 515, which are incorporated herein by reference. Alternatively, one may insert the sequences to be expressed in plant cells into a vector containing separate

replication sequences, one of which stabilizes the vector in *E. coli*, and the other in *Agrobacterium*. See, for example, McBride, *et al.* (*Plant Mol. Biol.* (1990) *14*:269-276), wherein the pRiHRI (Jouanin, *et al., Mol. Gen. Genet.* (1985) 201:370-374) origin of replication is utilized and provides for added stability of the plant expression vectors in host *Agrobacterium* cells.

Included with the expression construct and the T-DNA will be one or more markers, which allow for selection of transformed Agrobacterium and transformed plant cells. A number of markers have been developed for use with plant cells, such as resistance to chloramphenicol, kanamycin, the aminoglycoside G418, hygromycin, or the like. The particular marker employed is not essential to this invention, one or another marker being preferred depending on the particular host and the manner of construction.

For transformation of plant cells using *Agrobacterium*, explants may be combined and incubated with the transformed *Agrobacterium* for sufficient time for transformation, the bacteria killed, and the plant cells cultured in an appropriate selective medium. Once callus forms, shoot formation can be encouraged by employing the appropriate plant hormones in accordance with known methods and the shoots transferred to rooting medium for regeneration of plants. The plants may then be grown to seed and the seed used to establish repetitive generations and for isolation of vegetable oils.

There are several possible ways to obtain the plant cells of this invention which contain multiple expression constructs. Any means for producing a plant comprising a construct having a DNA sequence encoding the expression construct of the present invention, and at least one other construct having another DNA sequence encoding an enzyme are encompassed by the present invention. For example, the expression construct can be used to transform a plant at the same time as the second construct either by inclusion of both expression constructs in a single transformation vector or by using separate vectors, each of which express desired genes. The second construct can be introduced into a plant which has already been transformed with the prenyltransferase or tocopherol cyclase expression construct, or alternatively, transformed plants, one expressing the prenyltransferase or tocopherol cyclase construct and one expressing the second construct, can be crossed to bring the constructs together in the same plant.

Transgenic plants of the present invention may be produced from tissue culture, and subsequent generations grown from seed. Alternatively, transgenic plants may be grown using apomixis. Apomixis is a genetically controlled method of reproduction in plants where the embryo is formed without union of an egg and a sperm. There are three basic types of apomictic reproduction: 1) apospory where the embryo develops from a chromosomally unreduced egg in an embryo sac derived from the nucleus, 2) diplospory where the embryo develops from an unreduced egg in an embryo sac derived from the megaspore mother cell, and 3) adventitious embryony where the embryo develops directly from a somatic cell. In most forms of apomixis, pseudogamy or fertilization of the polar nuclei to produce endosperm is necessary for seed viability. In apospory, a nurse cultivar can be used as a pollen source for endosperm formation in seeds. The nurse cultivar does not affect the genetics of the aposporous apomictic cultivar since the unreduced egg of the cultivar develops parthenogenetically, but makes possible endosperm production. Apomixis is economically important, especially in transgenic plants, because it causes any genotype, no matter how heterozygous, to breed true. Thus,with apomictic reproduction, heterozygous transgenic plants can maintain their genetic fidelity throughout repeated life cycles. Methods for the production of apomictic plants are known in the art. See, U.S. Patent No.5,811,636, which is herein incorporated by reference in its entirety.

The nucleic acid sequences of the present invention can be used in constructs to provide for the expression of the sequence in a variety of host cells, both prokaryotic eukaryotic. Host cells of the present invention preferably include monocotyledenous and dicotyledenous plant cells.

In general, the skilled artisan is familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of macromolecules (e.g., DNA molecules, plasmids, etc.), generation of recombinant organisms and the screening and isolating of clones, (see for example, Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press (1989); Maliga *et al., Methods in Plant Molecular Biology,* Cold Spring Harbor Press (1995), the entirety of which is herein incorporated by reference; Birren *et al., Genome Analysis: Analyzing DNA,* 1, Cold Spring Harbor, New York, the entirety of which is herein incorporated by reference).

Methods for the expression of sequences in insect host cells are known in the art. Baculovirus expression vectors are recombinant insect viruses in which the coding sequence for a chosen foreign gene has been inserted behind a baculovirus promoter in place of the viral gene, e.g., polyhedrin (Smith and Summers, U.S. Pat. No., 4,745,051, the entirety of which is incorporated herein by reference). Baculovirus expression vectors are known in the art, and are described for example in Doerfler, *Curr. Top. Microbiol. Immunol. 131*:51-68 (1968); Luckow and Summers, *Bio/Technology* 6:47-55 (1988a); Miller, *Annual Review of Microbiol. 42*:177-199 (1988); Summers, *Curr. Comm. Molecular Biology*, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1988); Summers and Smith, *A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures,* Texas Ag. Exper. Station Bulletin No. 1555 (1988), the entireties of which is herein incorporated by reference)

Methods for the expression of a nucleic acid sequence of interest in a fungal host cell are known in the art. The fungal host cell may, for example, be a yeast cell or a filamentous fungal cell. Methods for the expression of DNA sequences of interest in yeast cells are generally described in “Guide to yeast genetics and molecular biology”, Guthrie and Fink, eds. Methods in enzymology , Academic Press, Inc. Vol 194 (1991) and Gene expression technology”, Goeddel ed, Methods in Enzymology, Academic Press, Inc., Vol 185 (1991).

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC, Manassas, VA), such as HeLa cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells and a number of other cell lines. Suitable promoters for mammalian cells are also known in the art and include, but are not limited to, viral promoters such as that from Simian Virus 40 (SV40) (Fiers *et al., Nature* 273:113 (1978), the entirety of which is herein incorporated by reference), Rous sarcoma virus (RSV), adenovirus (ADV) and bovine papilloma virus (BPV). Mammalian cells may also require terminator sequences and poly-A addition sequences. Enhancer sequences which increase expression may also be included and sequences which promote amplification of the gene may also be desirable (for example methotrexate resistance genes).

Vectors suitable for replication in mammalian cells are well known in the art, and may include viral replicons, or sequences which insure integration of the appropriate sequences

encoding epitopes into the host genome. Plasmid vectors that greatly facilitate the construction of recombinant viruses have been described (*see*, for example, Mackett *et al*, *J Virol. 49*:857 (1984); Chakrabarti *et al.*, *Mol. Cell. Biol. 5*:3403 (1985); Moss, In: *Gene Transfer Vectors For Mammalian Cells* (Miller and Calos, eds., Cold Spring Harbor Laboratory, N.Y., p. 10, (1987); all of which are herein incorporated by reference in their entirety).

The invention also includes plants and plant parts, such as seed, oil and meal derived from seed, and feed and food products processed from plants, which are enriched in tocopherols. Of particular interest is seed oil obtained from transgenic plants where the tocopherol level has been increased as compared to seed oil of a non-transgenic plant.

The harvested plant material may be subjected to additional processing to further enrich the tocopherol content. The skilled artisan will recognize that there are many such processes or methods for refining, bleaching and degumming oil. United States Patent Number 5,932,261, issued August 3, 1999, discloses on such process, for the production of a natural carotene rich refined and deodorised oil by subjecting the oil to a pressure of less than 0.060 mbar and to a temperature of less than 200.degree. C. Oil distilled by this process has reduced free fatty acids, yielding a refined, deodorised oil where Vitamin E contained in the feed oil is substantially retained in the processed oil. The teachings of this patent are incorporated herein by reference.

The invention now being generally described, it will be more readily understood by reference to the following examples which are included for purposes of illustration only and are not intended to limit the present invention.

EXAMPLES

Example 1: Identification of Prenyltransferase or tocopherol cyclase Sequences

PSI-BLAST (Altschul, *et al.* (1997) *Nuc Acid Res* 25:3389-3402) profiles were generated for both the straight chain and aromatic classes of prenyltransferases. To generate the straight chain profile, a prenyl- transferase from *Porphyra purpurea* (Genbank accession 1709766) was used as a query against the NCBI non-redundant protein database. The *E. coli* enzyme involved

in the formation of ubiquinone, ubiA (genbank accession 1790473) was used as a starting sequence to generate the aromatic prenyltransferase profile. These profiles were used to search public and proprietary DNA and protein data bases. In *Arabidopsis* six putative prenyltransferases of the straight-chain class were identified, ATPT1, (SEQ ID NO:9), ATPT7 (SEQ ID NO:10), ATPT8 (SEQ ID NO:11), ATPT9 (SEQ ID NO:13), ATPT10 (SEQ ID NO:14), and ATPT11 (SEQ ID NO:15), and six were identified of the aromatic class, ATPT2 (SEQ ID NO:1), ATPT3 (SEQ ID NO:3), ATPT4 (SEQ ID NO:5), ATPT5 (SEQ ID NO:7), ATPT6 (SEQ ID NO:8), and ATPT12 (SEQ ID NO:16). Additional prenyltransferase sequences from other plants related to the aromatic class of prenyltransferases, such as soy (SEQ ID NOs: 19-23, the deduced amino acid sequence of SEQ ID NO:23 is provided in SEQ ID NO:24) and maize (SEQ ID NOs:25-29, and 31) are also identified. The deduced amino acid sequence of ZMPT5 (SEQ ID NO:29) is provided in SEQ ID NO:30.

Searches are performed on a Silicon Graphics Unix computer using additional Bioaccellerator hardware and GenWeb software supplied by Compugen Ltd. This software and hardware enables the use of the Smith-Waterman algorithm in searching DNA and protein databases using profiles as queries. The program used to query protein databases is profilesearch. This is a search where the query is not a single sequence but a profile based on a multiple alignment of amino acid or nucleic acid sequences. The profile is used to query a sequence data set, i.e., a sequence database. The profile contains all the pertinent information for scoring each position in a sequence, in effect replacing the "scoring matrix" used for the standard query searches. The program used to query nucleotide databases with a protein profile is tprofilesearch. Tprofilesearch searches nucleic acid databases using an amino acid profile query. As the search is running, sequences in the database are translated to amino acid sequences in six reading frames. The output file for tprofilesearch is identical to the output file for profilesearch except for an additional column that indicates the frame in which the best alignment occurred.

The Smith-Waterman algorithm, (Smith and Waterman (1981) *supra*), is used to search for similarities between one sequence from the query and a group of sequences contained in the database. E score values as well as other sequence information, such as conserved peptide sequences are used to identify related sequences.

To obtain the entire coding region corresponding to the *Arabidopsis* prenyltransferase sequences, synthetic oligo-nucleotide primers are designed to amplify the 5' and 3' ends of partial cDNA clones containing prenyltransferase sequences. Primers are designed according to the respective *Arabidopsis* prenyltransferase sequences and used in Rapid Amplification of cDNA Ends (RACE) reactions (Frohman *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) using the Marathon cDNA amplification kit (Clontech Laboratories Inc, Palo Alto, CA).

Amino acid sequence alignments between ATPT2 (SEQ ID NO:2), ATPT3 (SEQ ID NO:4), ATPT4 (SEQ ID NO:6), ATPT8 (SEQ ID NO:12), and ATPT12 (SEQ ID NO:17) are performed using ClustalW (Figure 1), and the percent identity and similarities are provided in Table 1 below.

Table 1:

		ATPT2	ATPT3	ATPT4	ATPT8	ATPT12
ATPT2	% Identity		12	13	11	15
	% similar		25	25	22	32
	% Gap		17	20	20	9
ATPT3	% Identity			12	6	22
	% similar			29	16	38
	% Gap			20	24	14
ATPT4	% Identity				9	14
	% similar				18	29
	% Gap				26	19
ATPT8	% Identity					7
	% similar					19
	% Gap					20
ATPT12	% Identity					
	% similar					
	% Gap					

Example 2: Preparation of Prenyl Transferase Expression Constructs

A plasmid containing the napin cassette derived from pCGN3223 (described in USPN 5,639,790, the entirety of which is incorporated herein by reference) was modified to make it more useful for cloning large DNA fragments containing multiple restriction sites, and to allow the cloning of multiple napin fusion genes into plant binary transformation vectors. An adapter comprised of the self annealed oligonucleotide of sequence CGCGATTTAAATGGCGCGCCCTGCAGGCGGCCGCCTGCAGGGCGCGCCATTTAAAT (SEQ ID NO:40) was ligated into the cloning vector pBC SK+ (Stratagene) after digestion with the restriction endonuclease BssHII to construct vector pCGN7765. Plamids pCGN3223 and pCGN7765 were digested with NotI and ligated together. The resultant vector, pCGN7770, contains the pCGN7765 backbone with the napin seed specific expression cassette from pCGN3223.

The cloning cassette, pCGN7787, essentially the same regulatory elements as pCGN7770, with the exception of the napin regulatory regions of pCGN7770 have been replaced with the double CAMV 35S promoter and the tml polyadenylation and transcriptional termination region.

A binary vector for plant transformation, pCGN5139, was constructed from pCGN1558 (McBride and Summerfelt, (1990) Plant Molecular Biology, 14:269-276). The polylinker of pCGN1558 was replaced as a HindIII/Asp718 fragment with a polylinker containing unique restriction endonuclease sites, AscI, PacI, XbaI, SwaI, BamHI, and NotI. The Asp718 and HindIII restriction endonuclease sites are retained in pCGN5139.

A series of turbo binary vectors are constructed to allow for the rapid cloning of DNA sequences into binary vectors containing transcriptional initiation regions (promoters) and transcriptional termination regions.

The plasmid pCGN8618 was constructed by ligating oligonucleotides 5'-TCGAGGATCCGCGGCCGCAAGCTTCCTGCAGG-3' (SEQ ID NO:41) and 5'-TCGACCTGCAGGAAGCTTGCGGCCGCGGATCC-3' (SEQ ID NO:42) into SalI/XhoI-digested pCGN7770. A fragment containing the napin promoter, polylinker and napin 3' region was excised from pCGN8618 by digestion with Asp718I; the fragment was blunt-ended by filling in the 5' overhangs with Klenow fragment then ligated into pCGN5139 that had been digested with Asp718I and HindIII and blunt-ended by filling in the 5' overhangs with Klenow fragment.

A plasmid containing the insert oriented so that the napin promoter was closest to the blunted Asp718I site of pCGN5139 and the napin 3' was closest to the blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation and the integrity of cloning junctions. The resulting plasmid was designated pCGN8622.

The plasmid pCGN8619 was constructed by ligating oligonucleotides 5'-TCGACCTGCAGGAAGCTTGCGGCCGCGGATCC -3' (SEQ ID NO:43) and 5'-TCGAGGATCCGCGGCCGCAAGCTTCCTGCAGG-3' (SEQ ID NO:44) into SalI/XhoI-digested pCGN7770. A fragment containing the napin promoter, polylinker and napin 3' region was removed from pCGN8619 by digestion with Asp718I; the fragment was blunt-ended by filling in the 5' overhangs with Klenow fragment then ligated into pCGN5139 that had been digested with Asp718I and HindIII and blunt-ended by filling in the 5' overhangs with Klenow fragment. A plasmid containing the insert oriented so that the napin promoter was closest to the blunted Asp718I site of pCGN5139 and the napin 3' was closest to the blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation and the integrity of cloning junctions. The resulting plasmid was designated pCGN8623.

The plasmid pCGN8620 was constructed by ligating oligonucleotides 5'-TCGAGGATCCGCGGCCGCAAGCTTCCTGCAGGAGCT -3' (SEQ ID NO:45) and 5'-CCTGCAGGAAGCTTGCGGCCGCGGATCC-3' (SEQ ID NO:46) into SalI/SacI-digested pCGN7787. A fragment containing the d35S promoter, polylinker and tml 3' region was removed from pCGN8620 by complete digestion with Asp718I and partial digestion with NotI. The fragment was blunt-ended by filling in the 5' overhangs with Klenow fragment then ligated into pCGN5139 that had been digested with Asp718I and HindIII and blunt-ended by filling in the 5' overhangs with Klenow fragment. A plasmid containing the insert oriented so that the d35S promoter was closest to the blunted Asp718I site of pCGN5139 and the tml 3' was closest to the blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation and the integrity of cloning junctions. The resulting plasmid was designated pCGN8624.

The plasmid pCGN8621 was constructed by ligating oligonucleotides 5'-TCGACCTGCAGGAAGCTTGCGGCCGCGGATCCAGCT -3' (SEQ ID NO:47) and 5'-GGATCCGCGGCCGCAAGCTTCCTGCAGG-3' (SEQ ID NO:48) into SalI/SacI-digested

pCGN7787. A fragment containing the d35S promoter, polylinker and tml 3' region was removed from pCGN8621 by complete digestion with Asp718I and partial digestion with NotI. The fragment was blunt-ended by filling in the 5' overhangs with Klenow fragment then ligated into pCGN5139 that had been digested with Asp718I and HindIII and blunt-ended by filling in the 5' overhangs with Klenow fragment. A plasmid containing the insert oriented so that the d35S promoter was closest to the blunted Asp718I site of pCGN5139 and the tml 3' was closest to the blunted HindIII site was subjected to sequence analysis to confirm both the insert orientation and the integrity of cloning junctions. The resulting plasmid was designated pCGN8625.

The plasmid construct pCGN8640 is a modification of pCGN8624 described above. A 938bp PstI fragment isolated from transposon Tn7 which encodes bacterial spectinomycin and streptomycin resistance (Fling et al. (1985), *Nucleic Acids Research* 13(19):7095-7106), a determinant for E. coli and Agrobacterium selection, was blunt ended with Pfu polymerase. The blunt ended fragment was ligated into pCGN8624 that had been digested with SpeI and blunt ended with Pfu polymerase. The region containing the PstI fragment was sequenced to confirm both the insert orientation and the integrity of cloning junctions.

The spectinomycin resistance marker was introduced into pCGN8622 and pCGN8623 as follows. A 7.7 Kbp AvrII-SnaBI fragment from pCGN8640 was ligated to a 10.9 Kbp AvrII-SnaBI fragment from pCGN8623 or pCGN8622, described above. The resulting plasmids were pCGN8641 and pCGN8643, respectively.

The plasmid pCGN8644 was constructed by ligating oligonucleotides 5'-GATCACCTGCAGGAAGCTTGCGGCCGCGGATCCAATGCA-3' (SEQ ID NO:49) and 5'-TTGGATCCGCGGCCGCAAGCTTCCTGCAGGT-3' (SEQ ID NO:50) into BamHI-PstI digested pCGN8640.

Synthetic oligonulceotides were designed for use in Polymerase Chain Reactions (PCR) to amplify the coding sequences of ATPT2, ATPT3, ATPT4, ATPT8, and ATPT12 for the preparation of expression constructs and are provided in Table 2 below.

Table 2:

Name	Restriction Site	Sequence	SEQ ID NO:
ATPT2	5' NotI	GGATCCGCGGCCGCACAATGGAGTC TCTGCTCTCTAGTTCT	51

ATPT2	3' SseI	GGATCCTGCAGGTCACTTCAAAAAA GGTAACAGCAAGT	52
ATPT3	5' NotI	GGATCCGCGGCCGCACAATGGCGTT TTTTGGGCTCTCCCGTGTTT	53
ATPT3	3' SseI	GGATCCTGCAGGTTATTGAAAACTT CTTCCAAGTACAACT	54
ATPT4	5' NotI	GGATCCGCGGCCGCACAATGTGGCG AAGATCTGTTGTT	55
ATPT4	3' SseI	GGATCCTGCAGGTCATGGAGAGTAG AAGGAAGGAGCT	56
ATPT8	5' NotI	GGATCCGCGGCCGCACAATGGTACT TGCCGAGGTTCCAAAGCTTGCCTCT	57
ATPT8	3' SseI	GGATCCTGCAGGTCACTTGTTTCTG GTGATGACTCTAT	58
ATPT12	5' NotI	GGATCCGCGGCCGCACAATGACTTC GATTCTCAACACT	59
ATPT12	3' SseI	GGATCCTGCAGGTCAGTGTTGCGAT GCTAATGCCGT	60

The coding sequences of ATPT2, ATPT3, ATPT4, ATPT8, and ATPT12 were all amplified using the respective PCR primers shown in Table 2 above and cloned into the TopoTA vector (Invitrogen). Constructs containing the respective prenyltransferase sequences were digested with NotI and Sse8387I and cloned into the turbobinary vectors described above.

The sequence encoding ATPT2 prenyltransferase was cloned in the sense orientation into pCGN8640 to produce the plant transformation construct pCGN10800 (Figure 2). The ATPT2 sequence is under control of the 35S promoter.

The ATPT2 sequence was also cloned in the antisense orientation into the construct pCGN8641 to create pCGN10801 (Figure 3). This construct provides for the antisense expression of the ATPT2 sequence from the napin promoter.

The ATPT2 coding sequence was also cloned in the sense orientation into the vector pCGN8643 to create the plant transformation construct pCGN10822

The ATPT2 coding sequence was also cloned in the antisense orientation into the vector pCGN8644 to create the plant transformation construct pCGN10803 (Figure 4).

The ATPT4 coding sequence was cloned into the vector pCGN864 to create the plant transformation construct pCGN10806 (Figure 5). The ATPT2 coding sequence was cloned into the vector TopoTA ™ vector from Invitrogen, to create the plant transformation construct pCGN10807(Figure 6). The ATPT3 coding sequence was cloned into the TopoTA vector to create

the plant transformation construct pCGN10808 (Figure 7). The ATPT3 coding sequence was cloned in the sense orientation into the vector pCGN8640 to create the plant transformation construct pCGN10809 (Figure 8). The ATPT3 coding sequence was cloned in the antisense orientation into the vector pCGN8641 to create the plant transformation construct pCGN10810 (Figure 9). The ATPT3 coding sequence was cloned into the vector pCGN8643 to create the plant transformation construct pCGN10811 (Figure 10). The ATPT3 coding sequence was cloned into the vector pCGN8644 to create the plant transformation construct pCGN10812 (Figure 11). The ATPT4 coding sequence was cloned into the vector pCGN8640 to create the plant transformation construct pCGN10813 (Figure 12). The ATPT4 coding sequence was cloned into the vector pCGN8641 to create the plant transformation construct pCGN10814 (Figure 13). The ATPT4 coding sequence was cloned into the vector pCGN8643 to create the plant transformation construct pCGN10815 (Figure 14). The ATPT4 coding sequence was cloned in the antisense orientation into the vector pCGN8644 to create the plant transformation construct pCGN10816 (Figure 15). The ATPT8 coding sequence was cloned in the sense orientation into the vector pCGN8643 to create the plant transformation construct pCGN10819 (Figure 17). The ATPT12 coding sequence was cloned into the vector pCGN8640 to create the plant transformation construct pCGN10824 (Figure 18). The ATPT12 coding sequence was cloned into the vector pCGN8643 to create the plant transformation construct pCGN10825 (Figure 19). The ATPT8 coding sequence was cloned into the vector pCGN8640 to create the plant transformation construct pCGN10826 (Figure 20).

Example 3: Plant Transformation with Prenyl Transferase Constructs

Transgenic *Brassica* plants are obtained by *Agrobacterium*-mediated transformation as described by Radke *et al.* (*Theor. Appl. Genet.* (1988) *75*:685-694; *Plant Cell Reports* (1992) *11*:499-505). Transgenic *Arabidopsis thaliana* plants may be obtained by *Agrobacterium*-mediated transformation as described by Valverkens *et al.*, (*Proc. Nat. Acad. Sci.* (1988) *85*:5536-5540), or as described by Bent et al. ((1994), *Science* 265:1856-1860), or Bechtold et al. ((1993), *C.R.Acad.Sci, Life Sciences* 316:1194-1199). Other plant species may be similarly transformed using related techniques.

Alternatively, microprojectile bombardment methods, such as described by Klein *et al.* (*Bio/Technology 10*:286-291) may also be used to obtain nuclear transformed plants.

Example 4: Identification of Additional Prenyltransferases

Additional BLAST searches were performed using the ATPT2 sequence, a sequence in the class of aromatic prenyltransferases. ESTs, and in some case, full-length coding regions, were identified in proprietary DNA libraries.

Soy full-length homologs to ATPT2 were identified by a combination of BLAST (using ATPT2 protein sequence) and 5' RACE. Two homologs resulted (SEQ ID NO:95 and SEQ ID NO:96). Translated amino acid sequences are provided by SEQ ID NO:97 and SEQ ID NO:98.

A rice est ATPT2 homolog is shown in SEQ ID NO:99 (obtained from BLAST using the wheat ATPT2 homolog).

Other homolog sequences were obtained using ATPT2 and PSI-BLAST, including est sequences from wheat (SEQ ID NO:100), leek (SEQ ID NOs:101 and 102), canola (SEQ ID NO:103), corn (SEQ ID NOs:104, 105 and 106), cotton (SEQ ID NO:107) and tomato (SEQ ID NO:108).

A PSI-Blast profile generated using the *E. coli* ubiA (genbank accession 1790473) sequence was used to analyze the *Synechocystis* genome. This analysis identified 5 open reading frames (ORFs) in the *Synechocystis* genome that were potentially prenyltransferases; slr0926 (annotated as ubiA (4-hydroxybenzoate-octaprenyltransferase, SEQ ID NO:32), sll1899 (annotated as ctaB (cytocrome c oxidase folding protein, SEQ ID NO:33), slr0056 (annotated as g4 (chlorophyll synthase 33 kd subunit, SEQ ID NO:34), slr1518 (annotated as menA (menaquinone biosynthesis protein, SEQ ID NO:35), and slr1736 (annotated as a hypothetical protein of unknown function (SEQ ID NO:36).

4A. *Synechocystis* Knock-outs

To determine the functionality of these ORFs and their involvement, if any, in the biosynthesis of tocopherols, knockouts constructs were made to disrupt the ORF identified in *Synechocystis*.

Synthetic oligos were designed to amplify regions from the 5' (5'-TAATGTGTACATTGTCGGCCTC (17365') (SEQ ID NO:61) and 5'-GCAATGTAACATCAGAGATTTTGAGACACAACGTGGCTTTCCACAATTCCCCGCACC GTC (1736kanpr1)) (SEQ ID NO:62) and 3' (5'-AGGCTAATAAGCACAAATGGGA (17363') (SEQ ID NO:63) and 5'-GGTATGAGTCAGCAACACCTTCTTCACGAGGCAGACCTCAGC GGAATTGGTTTAGGTTATCCC (1736kanpr2)) (SEQ ID NO:64) ends of the slr1736 ORF. The 1736kanpr1 and 1736kanpr2 oligos contained 20 bp of homology to the slr1736 ORF with an additional 40 bp of sequence homology to the ends of the kanamycin resistance cassette. Separate PCR steps were completed with these oligos and the products were gel purified and combined with the kanamycin resistance gene from puc4K (Pharmacia) that had been digested with *Hinc*II and gel purified away from the vector backbone. The combined fragments were allowed to assemble without oligos under the following conditions: 94°C for 1 min, 55°C for 1 min, 72°C for 1 min plus 5 seconds per cycle for 40 cycles using pfu polymerase in 100ul reaction volume (Zhao, H and Arnold (1997) *Nucleic Acids Res.* 25(6):1307-1308). One microliter or five microliters of this assembly reaction was then amplified using 5' and 3' oligos nested within the ends of the ORF fragment, so that the resulting product contained 100-200 bp of the 5' end of the *Synechocystis* gene to be knocked out, the kanamycin resistance cassette, and 100-200 bp of the 3' end of the gene to be knocked out. This PCR product was then cloned into the vector pGemT easy (Promega) to create the construct pMON21681 and used for *Synechocystis* transformation.

Primers were also synthesized for the preparation of *Synechocystis* knockout constructs for the other sequences using the same method as described above, with the following primers. The ubiA 5' sequence was amplified using the primers 5'- GGATCCATGGTT GCCCAAACCCCATC (SEQ ID NO:65) and 5'- GCAATGTAACATCAGAGA TTTTGAGACACAACG TGGCTTTGGGTAAGCAACAATGACCGGC (SEQ ID NO:66). The 3' region was amplified using the synthetic oligonucleotide primers 5'-GAATTCTCAAAGCCAGCCCAGTAAC (SEQ ID NO:67) and 5'-GGTATGAGTC AGCAACACCTTCTTCACGAGGCAGACCTCAGCGGGTGCGAAAAGGGTTTTCCC (SEQ ID NO:68). The amplification products were combined with the kanamycin resistance gene from puc4K (Pharmacia) that had been digested with *Hinc*II and gel purified away from the vector

backbone. The annealed fragment was amplified using 5' and 3' oligos nested within the ends of the ORF fragment (5'- CCAGTGGTTTAGGCTGTGTGGTC (SEQ ID NO:69) and 5'- CTGAGTTGGATGTATTGGATC (SEQ ID NO:70)), so that the resulting product contained 100-200 bp of the 5' end of the *Synechocystis* gene to be knocked out, the kanamycin resistance cassette, and 100-200 bp of the 3' end of the gene to be knocked out. This PCR product was then cloned into the vector pGemT easy (Promega) to create the construct pMON21682 and used for *Synechocystis* transformation.

Primers were also synthesized for the preparation of *Synechocystis* knockout constructs for the other sequences using the same method as described above, with the following primers. The sll1899 5' sequence was amplified using the primers 5'- GGATCCATGGTTACTT CGACAAAAATCC (SEQ ID NO:71) and 5'- GCAATGTAACATCAGAG ATTTTGAGACACAACGTGGCTTTGCTAGGCAACCGCTTAGTAC (SEQ ID NO:72). The 3' region was amplified using the synthetic oligonucleotide primers 5'- GAATTCTTAACCCAACAGTAAAGTTCCC (SEQ ID NO:73) and 5'- GGTATGAGTCAGC AACACCTTCTTCACGAGGCAGACCTCAGCGCCGGCATTGTCTTTTACATG (SEQ ID NO:74). The amplification products were combined with the kanamycin resistance gene from puc4K (Pharmacia) that had been digested with *Hinc*II and gel purified away from the vector backbone. The annealed fragment was amplified using 5' and 3' oligos nested within the ends of the ORF fragment (5'- GGAACCCTTGCAGCCGCTTC (SEQ ID NO:75) and 5'- GTATGCCCAACTGGTGCAGAGG (SEQ ID NO:76)), so that the resulting product contained 100-200 bp of the 5' end of the *Synechocystis* gene to be knocked out, the kanamycin resistance cassette, and 100-200 bp of the 3' end of the gene to be knocked out. This PCR product was then cloned into the vector pGemT easy (Promega) to create the construct pMON21679 and used for *Synechocystis* transformation.

Primers were also synthesized for the preparation of *Synechocystis* knockout constructs for the other sequences using the same method as described above, with the following primers. The slr0056 5' sequence was amplified using the primers 5'- GGATCCATGTCTGACACACAAAATACCG (SEQ ID NO:77) and 5'- GCAATGTAACATCAGAGATTTTGAGACACAACGTGGCTTTCGCCAATACCAGCCACC AACAG (SEQ ID NO:78). The 3' region was amplified using the synthetic oligonucleotide

primers 5'- GAATTCTCAAAT CCCCGCATGGCCTAG (SEQ ID NO:79) and 5'-GGTATGAGTCAGCAACACCTTCTTCACGAGGCAGACCTCAGCGGCCTACGGCTTGGACGTGTGGG (SEQ ID NO:80). The amplification products were combined with the kanamycin resistance gene from puc4K (Pharmacia) that had been digested with *Hinc*II and gel purified away from the vector backbone. The annealed fragment was amplified using 5' and 3' oligos nested within the ends of the ORF fragment (5'- CACTTGGATTCCCCTGATCTG (SEQ ID NO:81) and 5'- GCAATACCCGCTTGGAAAACG (SEQ ID NO:82)), so that the resulting product contained 100-200 bp of the 5' end of the *Synechocystis* gene to be knocked out, the kanamycin resistance cassette, and 100-200 bp of the 3' end of the gene to be knocked out. This PCR product was then cloned into the vector pGemT easy (Promega) to create the construct pMON21677 and used for *Synechocystis* transformation.

Primers were also synthesized for the preparation of *Synechocystis* knockout constructs for the other sequences using the same method as described above, with the following primers. The slr1518 5' sequence was amplified using the primers 5'- GGATCCATGACCGAAT CTTCGCCCCTAGC (SEQ ID NO:83) and 5'-GCAATGTAACATCAGAGATTTTGA GACACAACGTGGC TTTCAATCCTAGGTAGCCGAGGCG (SEQ ID NO:84). The 3' region was amplified using the synthetic oligonucleotide primers 5'- GAATTCTTAGCCCAGGCC AGCCCAGCC (SEQ ID NO:85)and 5'- GGTATGAGTCAGCAACACCTTCTTCACGA GGCAGACCTCAGCGGGGAATTGATTTGTTTAATTACC (SEQ ID NO:86). The amplification products were combined with the kanamycin resistance gene from puc4K (Pharmacia) that had been digested with *Hinc*II and gel purified away from the vector backbone. The annealed fragment was amplified using 5' and 3' oligos nested within the ends of the ORF fragment (5'- GCGATCGCCATTATCGCTTGG (SEQ ID NO:87) and 5'-GCAGACTGGCAATTATCAGTAACG (SEQ ID NO:88)), so that the resulting product contained 100-200 bp of the 5' end of the *Synechocystis* gene to be knocked out, the kanamycin resistance cassette, and 100-200 bp of the 3' end of the gene to be knocked out. This PCR product was then cloned into the vector pGemT easy (Promega) to create the construct pMON21680 and used for *Synechocystis* transformation.

4B. Transformation of *Synechocystis*

Cells of *Synechocystis* 6803 were grown to a density of approximately $2x10^8$ cells per ml and harvested by centrifugation. The cell pellet was re-suspended in fresh BG-11 medium (ATCC Medium 616) at a density of $1x10^9$ cells per ml and used immediately for transformation. One-hundred microliters of these cells were mixed with 5 ul of mini prep DNA and incubated with light at 30C for 4 hours. This mixture was then plated onto nylon filters resting on BG-11 agar supplemented with TES pH8 and allowed to grow for 12-18 hours. The filters were then transferred to BG-11 agar + TES + 5ug/ml kanamycin and allowed to grow until colonies appeared within 7-10 days (Packer and Glazer, 1988). Colonies were then picked into BG-11 liquid media containing 5 ug/ml kanamycin and allowed to grow for 5 days. These cells were then transferred to Bg-11 media containing 10ug/ml kanamycin and allowed to grow for 5 days and then transferred to Bg-11 + kanamycin at 25ug/ml and allowed to grow for 5 days. Cells were then harvested for PCR analysis to determine the presence of a disrupted ORF and also for HPLC analysis to determine if the disruption had any effect on tocopherol levels.

PCR analysis of the *Synechocystis* isolates for slr1736 and sll1899 showed complete segregation of the mutant genome, meaning no copies of the wild type genome could be detected in these strains. This suggests that function of the native gene is not essential for cell function. HPLC analysis of these same isolates showed that the sll1899 strain had no detectable reduction in tocopherol levels. However, the strain carrying the knockout for slr1736 produced no detectable levels of tocopherol.

The amino acid sequences for the *Synechocystis* knockouts are compared using ClustalW, and are provided in Table 3 below. Provided are the percent identities, percent similarity, and the percent gap. The alignment of the sequences is provided in Figure 21.

Table 3:

	Slr1736	slr0926	sll1899	slr0056	slr1518
slr1736 %identity		14	12	18	11
%similar		29	30	34	26
%gap		8	7	10	5
slr0926 %identity			20	19	14
%similar			39	32	28

%gap	7	9	4
sll1899 %identity		17	13
%similar		29	29
%gap		12	9
slr0056 %identity			15
%similar			31
%gap			8
slr1518 %identity			
%similar			
%gap			

Amino acid sequence comparisons are performed using various *Arabidopsis* prenyltransferase sequences and the *Synechocystis* sequences. The comparisons are presented in Table 4 below. Provided are the percent identities, percent similarity, and the percent gap. The alignment of the sequences is provided in Figure 22.

Table 4:

	ATPT2	slr1736	ATPT3	slr0926	ATPT4	sll1899	ATPT12	slr0056	ATPT8	slr1518
ATPT2		29	9	9	8	8	12	9	7	9
		46	23	21	20	20	28	23	21	20
		27	13	28	23	29	11	24	25	24
slr1736			9	13	8	12	13	15	8	10
			19	28	19	28	26	33	21	26
			34	12	34	15	26	10	12	10
ATPT3				23	11	14	13	10	5	11
				36	26	26	26	21	14	22
				29	21	31	16	30	30	30
					12	20	17	20	11	14
slr0926					24	37	28	33	24	29
					33	12	25	10	11	9

	18	11	8	6	7
ATPT4	33	23	18	16	19
	28	19	32	32	33
		13	17	10	12
sll1899		24	30	23	26
		27	13	10	11
			52	8	11
ATPT12			66	19	26
			18	25	23
				9	13
slr0056				23	32
				10	8
					7
ATPT8					23
					7
slr1518					

4C. Phytyl Prenyltransferase Enzyme Assays

[^{3}H] Homogentisic acid in 0.1% H_3PO_4 (specific radioactivity 40 Ci/mmol). Phytyl pyrophosphate was synthesized as described by Joo, *et al.* (1973) *Can J. Biochem.* 51:1527. 2-methyl-6-phytylquinol and 2,3-dimethyl-5-phytylquinol were synthesized as described by Soll, *et al.* (1980) *Phytochemistry* 19:215. Homogentisic acid, α, β, δ, and γ-tocopherol, and tocol, were purchased commercially.

The wild-type strain of *Synechocystis* sp. PCC 6803 was grown in BG11 medium with bubbling air at 30°C under 50 $\mu E.m^{-2}.s^{-1}$ fluorescent light, and 70% relative humidity. The growth medium of slr1736 knock-out (potential PPT) strain of this organism was supplemented with 25

μg mL^{-1} kanamycin. Cells were collected from 0.25 to 1 liter culture by centrifugation at 5000 ***g*** for 10 min and stored at -80°C.

Total membranes were isolated according to Zak's procedures with some modifications (Zak, *et al.* (1999) *Eur J. Biochem* 261:311). Cells were broken on a French press. Before the French press treatment, the cells were incubated for 1 hour with lysozyme (0.5%, w/v) at 30 °C in a medium containing 7 mM EDTA, 5 mM NaCl and 10 mM Hepes-NaOH, pH 7.4. The spheroplasts were collected by centrifugation at 5000 ***g*** for 10 min and resuspended at 0.1 - 0.5 mg chlorophyll·mL^{-1} in 20 mM potassium phosphate buffer, pH 7.8. Proper amount of protease inhibitor cocktail and DNAase I from Boehringer Mannheim were added to the solution. French press treatments were performed two to three times at 100 MPa. After breakage, the cell suspension was centrifuged for 10 min at 5000g to pellet unbroken cells, and this was followed by centrifugation at 100 000 ***g*** for 1 hour to collect total membranes. The final pellet was resuspended in a buffer containing 50 mM Tris-HCL and 4 mM $MgCl_2$.

Chloroplast pellets were isolated from 250 g of spinach leaves obtained from local markets. Devined leaf sections were cut into grinding buffer (2 l /250 g leaves) containing 2 mM EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 0.33 M sorbitol, 0.1% ascorbic acid, and 50 mM Hepes at pH 7.5. The leaves were homogenized for 3 sec three times in a 1-L blendor, and filtered through 4 layers of mirocloth. The supernatant was then centrifuged at 5000***g*** for 6 min. The chloroplast pellets were resuspended in small amount of grinding buffer (Douce,*et al* Methods in Chloroplast Molecular Biology, 239 (1982)

Chloroplasts in pellets can be broken in three ways. Chloroplast pellets were first aliquoted in 1 mg of chlorophyll per tube, centrifuged at 6000 rpm for 2 min in microcentrifuge, and grinding buffer was removed. Two hundred microliters of Triton X-100 buffer (0.1% Triton X-100, 50 mM Tris-HCl pH 7.6 and 4 mM $MgCl_2$) or swelling buffer (10 mM Tris pH 7.6 and 4 mM $MgCl_2$) was added to each tube and incubated for ½ hour at 4°C. Then the broken chloroplast pellets were used for the assay immediately. In addition, broken chloroplasts can also be obtained by freezing in liquid nitrogen and stored at -80°C for ½ hour, then used for the assay.

In some cases chloroplast pellets were further purified with 40%/ 80% percoll gradient to obtain intact chloroplasts. The intact chloroplasts were broken with swelling buffer, then either used for assay or further purified for envelope membranes with 20.5%/ 31.8% sucrose density

gradient (Sol, *et al* (1980) *supra*). The membrane fractions were centrifuged at 100 000g for 40 min and resuspended in 50 mM Tris-HCl pH 7.6, 4 mM $MgCl_2$.

Various amounts of [^{3}H]HGA, 40 to 60 µM unlabelled HGA with specific activity in the range of 0.16 to 4 Ci/mmole were mixed with a proper amount of 1M Tris-NaOH pH 10 to adjust pH to 7.6. HGA was reduced for 4 min with a trace amount of solid $NaBH_4$. In addition to HGA, standard incubation mixture (final vol 1 mL) contained 50 mM Tris-HCl, pH 7.6, 3-5 mM $MgCl_2$, and 100 µM phytyl pyrophosphate. The reaction was initiated by addition of *Synechocystis* total membranes, spinach chloroplast pellets, spinach broken chloroplasts, or spinach envelope membranes. The enzyme reaction was carried out for 2 hour at 23°C or 30°C in the dark or light. The reaction is stopped by freezing with liquid nitrogen, and stored at -80°C or directly by extraction.

A constant amount of tocol was added to each assay mixture and reaction products were extracted with a 2 mL mixture of chloroform/methanol (1:2, v/v) to give a monophasic solution. NaCl solution (2 mL; 0.9%) was added with vigorous shaking. This extraction procedure was repeated three times. The organic layer containing the prenylquinones was filtered through a 20 mµ filter, evaporated under N_2, and then resuspended in 100 µL of ethanol.

The samples were mainly analyzed by Normal-Phase HPLC method (Isocratic 90% Hexane and 10% Methyl-t-butyl ether), and use a Zorbax silica column, 4.6 x 250 mm. The samples were also analyzed by Reversed-Phase HPLC method (Isocratic 0.1% H_3PO_4 in MeOH), and use a Vydac 201HS54 C18 column; 4.6 x 250 mm coupled with an All-tech C18 guard column. The amount of products were calculated based on the substrate specific radioactivity, and adjusted according to the % recovery based on the amount of internal standard.

The amount of chlorophyll was determined as described in Arnon (1949) *Plant Physiol.* 24:1. Amount of protein was determined by the Bradford method using gamma globulin as a standard (Bradford, (1976) *Anal. Biochem.* 72:248)

Results of the assay demonstrate that 2-Methyl-6-Phytylplastoquinone is not produced in the *Synechocystis* slr1736 knockout preparations. The results of the phytyl prenyltransferase enzyme activity assay for the slr1736 knock out are presented in Figure 23.

4D. Complementation of the slr1736 knockout with ATPT2

In order to determine whether ATPT2 could complement the knockout of slr1736 in *Synechocystis 6803*, a plasmid was constructed to express the ATPT2 sequence from the TAC promoter. A vector, plasmid psl1211, was obtained from the lab of Dr. Himadri Pakrasi of Washington University, and is based on the plasmid RSF1010 which is a broad host range plasmid (Ng W.-O., Zentella R., Wang, Y., Taylor J-S. A., Pakrasi, H.B. 2000. *phrA*, the major photoreactivating factor in the cyanobacterium *Synechocystis* sp. strain PCC 6803 codes for a cyclobutane pyrimidine dimer specific DNA photolyase. *Arch. Microbiol.* (in press)). The ATPT2 gene was isolated from the vector pCGN10817 by PCR using the following primers. ATPT2nco.pr 5'-CCATGGATTCGAGTAAAGTTGTCGC (SEQ ID NO:89); ATPT2ri.pr- 5'-GAATTCACTTCAAAAAAGGTAACAG (SEQ ID NO:90). These primers will remove approximately 112 BP from the 5' end of the ATPT2 sequence, which is thought to be the chloroplast transit peptide. These primers will also add an NcoI site at the 5' end and an EcoRI site at the 3' end which can be used for sub-cloning into subsequent vectors. The PCR product from using these primers and pCGN10817 was ligated into pGEM T easy and the resulting vector pMON21689 was confirmed by sequencing using the m13forward and m13reverse primers. The NcoI/EcoRI fragment from pMON21689 was then ligated with the EagI/EcoRI and EagI/NcoI fragments from psl1211 resulting in pMON21690. The plasmid pMON21690 was introduced into the slr1736 *Synechocystis 6803* KO strain via conjugation. Cells of sl906 (a helper strain) and DH10B cells containing pMON21690 were grown to log phase (O.D. 600= 0.4) and 1 ml was harvested by centrifugation. The cell pellets were washed twice with a sterile BG-11 solution and resuspended in 200 ul of BG-11. The following was mixed in a sterile eppendorf tube: 50 ul SL906, 50 ul DH10B cells containing pMON21690, and 100 ul of a fresh culture of the slr1736 *Synechocystis 6803* KO strain (O.D. 730 = 0.2-0.4). The cell mixture was immediately transferred to a nitrocellulose filter resting on BG-11 and incubated for 24 hours at 30C and 2500 LUX(50 ue) of light. The filter was then transferred to BG-11 supplemented with 10ug/ml Gentamycin and incubated as above for ~5 days. When colonies appeared, they were picked and grown up in liquid BG-11 + Gentamycin 10 ug/ml. (Elhai, J. and Wolk, P. 1988. Conjugal transfer of DNA to Cyanobacteria. *Methods in Enzymology* 167, 747-54) The liquid cultures were then assayed for tocopherols by harvesting 1ml of culture by centrifugation, extracting with ethanol/pyrogallol, and HPLC separation. The slr1736 *Synechocystis 6803* KO

strain, did not contain any detectable tocopherols, while the slr1736 *Synechocystis 6803* KO strain transformed with pmon21690 contained detectable alpha tocopherol. A *Synechocystis 6803* strain transformed with psl1211(vector control) produced alpha tocopherol as well.

4E: Additional Evidence of Prenyltransferase Activity

To test the hypothesis that slr1736 or ATPT2 are sufficient as single genes to obtain phytyl prenyltransferase activity, both genes were expressed in SF9 cells and in yeast. When either slr1736 or ATPT2 were expressed in insect cells (Table 5) or in yeast, phytyl prenyltransferase activity was detectable in membrane preparations, whereas membrane preparations of the yeast vector control, or membrane preparations of insect cells did not exhibit phytyl prenyltransferase activity.

Table 5: Phytyl prenyltransferase activity

Enzyme source	Enzyme activity [pmol/mg x h]
slr1736 expressed in SF9 cells	20
ATPT2 expressed in SF9 cells	6
SF9 cell control	< 0.05
Synechocystis 6803	0.25
Spinach chloroplasts	0.20

Example 5: Transgenic Plant Analysis

5A. *Arabidopsis*

Arabidopsis plants transformed with constructs for the sense or antisense expression of the ATPT proteins were analyzed by High Pressure Liquid Chromatography (HPLC) for altered levels of total tocopherols, as well as altered levels of specific tocopherols (alpha, beta, gamma, and delta tocopherol).

Extracts of leaves and seeds were prepared for HPLC as follows. For seed extracts, 10 mg of seed was added to 1 g of microbeads (Biospec) in a sterile microfuge tube to which 500 ul 1% pyrogallol (Sigma Chem)/ethanol was added. The mixture was shaken for 3 minutes in a

mini Beadbeater (Biospec) on "fast" speed. The extract was filtered through a 0.2 um filter into an autosampler tube. The filtered extracts were then used in HPLC analysis described below.

Leaf extracts were prepared by mixing 30-50 mg of leaf tissue with 1 g microbeads and freezing in liquid nitrogen until extraction. For extraction, 500 ul 1% pyrogallol in ethanol was added to the leaf/bead mixture and shaken for 1 minute on a Beadbeater (Biospec) on "fast" speed. The resulting mixture was centrifuged for 4 minutes at 14,000 rpm and filtered as described above prior to HPLC analysis.

HPLC was performed on a Zorbax silica HPLC column (4.6 mm X 250 mm) with a fluorescent detection, an excitation at 290 nm, an emission at 336 nm, and bandpass and slits. Solvent A was hexane and solvent B was methyl-t-butyl ether. The injection volume was 20 ul, the flow rate was 1.5 ml/min, the run time was 12 min (40°C) using the gradient (Table 6):

Table 6:

Time	Solvent A	Solvent B
0 min.	90%	10%
10 min.	90%	10%
11 min.	25%	75%
12 min.	90%	10%

Tocopherol standards in 1% pyrogallol/ ethanol were also run for comparison (alpha tocopherol, gamma tocopherol, beta tocopherol, delta tocopherol, and tocopherol (tocol) (all from Matreya).

Standard curves for alpha, beta, delta, and gamma tocopherol were calculated using Chemstation software. The absolute amount of component x is: Absolute amount of x= $Response_x$ x RF_x x dilution factor where $Response_x$ is the area of peak x, RF_x is the response factor for component x ($Amount_x/Response_x$) and the dilution factor is 500 ul. The ng/mg tissue is found by: total ng component/mg plant tissue.

Results of the HPLC analysis of seed extracts of transgenic *Arabidopsis* lines containing pMON10822 for the expression of ATPT2 from the napin promoter are provided in Figure 24.

HPLC analysis results of segregating T2 *Arabidopsis* seed tissue expressing the ATPT2 sequence from the napin promoter (pCGN10822) demonstrates an increased level of tocopherols in the seed. Total tocopherol levels are increased as much as 50% over the total tocopherol levels of non-transformed (wild-type) *Arabidopsis* plants (Figure 25). Homozygous progeny from the top 3 lines (T3 seed) have up to a two-fold (100%) increase in total tocopherol levels over control *Arabidopsis* seed (Figure 26.)

Furthermore, increases of particular tocopherols are also increased in transgenic *Arabidopsis* plants expressing the ATPT2 nucleic acid sequence from the napin promoter. Levels of delta tocopherol in these lines are increased greater than 3 fold over the delta tocopherol levels obtained from the seeds of wild type *Arabidopsis* lines. Levels of gamma tocopherol in transgenic *Arabidopsis* lines expressing the ATPT2 nucleic acid sequence are increased as much as about 60% over the levels obtained in the seeds of non-transgenic control lines. Furthermore, levels of alpha tocopherol are increased as much as 3 fold over those obtained from non-transgenic control lines.

Results of the HPLC analysis of seed extracts of transgenic *Arabidopsis* lines containing pCGN10803 for the expression of ATPT2 from the enhanced 35S promoter (antisense orientation) are provided in Figure 25. Two lines were identified that have reduced total tocopherols, up to a ten-fold decrease observed in T3 seed compared to control *Arabidopsis* (Figure 27.)

5B. Canola

Brassica napus, variety SP30021, was transformed with pCGN10822 (napin-ATPT2-napin 3', sense orientation) using *Agrobacterium tumefaciens*-mediated transformation. Flowers of the R0 plants were tagged upon pollination and developing seed was collected at 35 and 45 days after pollination (DAP).

Developing seed was assayed for tocopherol levels, as described above for *Arabidopsis*. Line 10822-1 shows a 20% increase of total tocopherols, compared to the wild-type control, at 45 DAP. Figure 28 shows total tocopherol levels measured in developing canola seed.

Example 6: Sequences to Tocopherol Cyclase

6A. Preparation of the slr1737 Knockout

The *Synechocystis sp. 6803* slr1737 knockout was constructed by the following method. The GPS™-1 Genome Priming System (New England Biolabs) was used to insert, by a Tn7 Transposase system, a Kanamycin resistance cassette into *slr1737*. A plasmid from a *Synechocystis* genomic library clone containing 652 base pairs of the targeted orf (*Synechcocystis* genome base pairs 1324051 – 1324703; the predicted orf base pairs 1323672 – 1324763, as annotated by Cyanobase) was used as target DNA. The reaction was performed according to the manufacturers protocol. The reaction mixture was then transformed into *E. coli* DH10B electrocompetant cells and plated. Colonies from this transformation were then screened for transposon insertions into the target sequence by amplifying with M13 Forward and Reverse Universal primers, yielding a product of 652 base pairs plus ~1700 base pairs, the size of the transposon kanamycin cassette, for a total fragment size of ~2300 base pairs. After this determination, it was then necessary to determine the approximate location of the insertion within the targeted orf, as 100 base pairs of orf sequence was estimated as necessary for efficient homologous recombination in *Synechocystis*. This was accomplished through amplification reactions using either of the primers to the ends of the transposon, Primer S (5' end) or N (3' end), in combination with either a M13 Forward or Reverse primer. That is, four different primer combinations were used to map each potential knockout construct: Primer S – M13 Forward, Primer S – M13 Reverse, Primer N – M13 Forward, Primer N – M13 Reverse. The construct used to transform *Synechocystis* and knockout slr1737 was determined to consist of a approximately 150 base pairs of slr1737 sequence on the 5' side of the transposon insertion and approximately 500 base pairs on the 3' side, with the transcription of the orf and kanamycin cassette in the same direction. The nucleic acid sequence of slr1737 is provided in SEQ ID NO:38 the deduced amino acid sequence is provided in SEQ ID NO:39.

Cells of *Synechocystis 6803* were grown to a density of ~ $2x10^8$ cells per ml and harvested by centrifugation. The cell pellet was re-suspended in fresh BG-11 medium at a density of $1x10^9$ cells per ml and used immediately for transformation. 100 ul of these cells were mixed with 5 ul of mini prep DNA and incubated with light at 30C for 4 hours. This mixture was then plated onto nylon filters resting on BG-11 agar supplemented with TES ph8 and allowed to grow for 12-18 hours. The filters were then transferred to BG-11 agar + TES +

5ug/ml kanamycin and allowed to grow until colonies appeared within 7-10 days (Packer and Glazer, 1988). Colonies were then picked into BG-11 liquid media containing 5 ug/ml kanamycin and allowed to grow for 5 days. These cells were then transferred to Bg-11 media containing 10ug/ml kanamycin and allowed to grow for 5 days and then transferred to Bg-11 + kanamycin at 25ug/ml and allowed to grow for 5 days. Cells were then harvested for PCR analysis to determine the presence of a disrupted ORF and also for HPLC analysis to determine if the disruption had any effect on tocopherol levels.

PCR analysis of the *Synechocystis* isolates, using primers to the ends of the *slr1737* orf , showed complete segregation of the mutant genome, meaning no copies of the wild type genome could be detected in these strains. This suggests that function of the native gene is not essential for cell function. HPLC analysis of the strain carrying the knockout for *slr1737* produced no detectable levels of tocopherol.

6B. The relation of slr1737 and slr1736

The slr1737 gene occurs in *Synechocystis* downstream and in the same orientation as slr1736, the phytyl prenyltransferase. In bacteria this proximity often indicates an operon structure and therefore an expression pattern that is linked in all genes belonging to this operon. Occasionally such operons contain several genes that are required to constitute one enzyme. To confirm that slr1737 is not required for phytyl prenyltransferase activity, phytyl prenyltransferase was measured in extracts from the *Synechocystis* slr1737 knockout mutant. Figure 29 shows that extracts from the *Synechocystis* slr1737 knockout mutant still contain phytyl prenyltransferase activity. The molecular organization of genes in *Synechocystis* 6803 is shown in A. Figures B and C show HPLC traces (normal phase HPLC) of reaction products obtained with membrane preparations from *Synechocystis* wild type and slr1737$^-$ membrane preparations, respectively.

The fact that slr1737 is not required for the PPT activity provides additional data that ATPT2 and slr1736 encode phytyl prenyltransferases.

6C *Synechocystis* Knockouts

Synechocystis 6803 wild type and *Synechocystis* slr1737 knockout mutant were grown photoautotrophically. Cells from a 20 ml culture of the late logarithmic growth phase were

harvested and extracted with ethanol. Extracts were separated by isocratic normal-phase HPLC using a Hexane/Methyl-t-butyl ether (95/5) and a Zorbax silica column, 4.6 x 250 mm. Tocopherols and tocopherol intermediates were detected by fluorescence (excitement 290 nm, emission 336 nm) (Figure 30).

Extracts of *Synechocystis* 6803 contained a clear signal of alpha-tocopherol. 2,3-Dimethyl-5-phytylplastoquinol was below the limit of detection in extracts from the *Synechocystis* wild type (C). In contrast, extracts from the *Synechocystis* slr1737 knockout mutant did not contain alpha-tocopherol, but contained 2,3-dimethyl-5-phytylplastoquinol (D), indicating that the interruption of slr1737 has resulted in a block of the 2,3-dimethyl-5-phytylplastoquinol cyclase reaction.

Chromatograms of standard compounds alpha, beta, gamma, delta-tocopherol and 2,3-dimethyl-5-phytylplastoquinol are shown in A and B. Chromatograms of extracts form *Synechocystis* wild type and the *Synechocystis* slr1737 knockout mutant are shown in C and D, respectively. Abbreviations: 2,3-DMPQ, 2,3-dimethyl-5-phytylplastoquinol.

6D. Incubation with Lysozyme treated *Synechocystis*

Synechocystis 6803 wild type and slr1737 knockout mutant cells from the late logarithmic growth phase (approximately 1g wet cells per experiment in a total volume of 3 ml) were treated with Lysozyme and subsequently incubated with S-adenosylmethionine, and phytylpyrophosphate, plus radiolabelled homogentisic acid. After 17h incubation in the dark at room temperature the samples were extracted with 6 ml chloroform / methanol (1/2 v/v). Phase separation was obtained by the addition of 6 ml 0.9% NaCl solution. This procedure was repeated three times. Under these conditions 2,3-dimethyl-5-phytylplastoquinol is oxidized to form 2,3-dimethyl-5-phytylplastoquinone.

The extracts were analyzed by normal phase and reverse phase HPLC. Using extracts from wild type *Synechocystis* cells radiolabelled gamma-tocopherol and traces of radiolabelled 2,3-dimethyl-5-phytylplastoquinone were detected. When extracts from the slr1737 knockout mutant were analyzed, only radiolabelled 2,3-dimethyl-5-phytylplastoquinone was detectable. The amount of 2,3-dimethyl-5-phytylplastoquinone was significantly increased compared to wild type extracts. Heat treated samples of the wild type and the slr1737 knockout mutant did not

produce radiolabelled 2,3-dimethyl-5-phytylplastoquinone, nor radiolabelled tocopherols. These results further support the role of the slr1737 expression product in the cyclization of 2,3-dimethyl-5-phytylplastoquinol.

6E. *Arabidopsis* Homologue to slr1737

An *Arabidopsis* homologue to slr1737 was identified from a BLASTALL search using *Synechocystis* sp 6803 gene slr1737 as the query, in both public and proprietary databases. SEQ ID NO:109 and SEQ ID NO:110 are the DNA and translated amino acid sequences, respectively, of the *Arabidopsis* homologue to slr1737. The start if found at the ATG at base 56 in SEQ ID NO:109.

The sequences obtained for the homologue from the proprietary database differs from the public database (F4D11.30, BAC AL022537), in having a start site 471 base pairs upstream of the start identified in the public sequence. A comparison of the public and proprietary sequences is provided in Figure 31. The correct start correlates within the public database sequence is at 12080, while the public sequence start is given as being at 11609.

Attempts to amplify a slr1737 homologue were unsuccessful using primers designed from the public database, while amplification of the gene was accomplished with primers obtained from SEQ ID NO:109.

Analysis of the protein sequence to identify transit peptide sequence predicted two potential cleavage sites, one between amino acids 48 and 49, and the other between amino acids 98 and 99.

6F. slr1737 Protein Information

The slr1737 orf comprises 363 amino acid residues and has a predicted MW of 41kDa (SEQ ID NO: 39). Hydropathic analysis indicates the protein is hydrophillic (Figure 32).

The *Arabidopsis* homologue to slr1737 (SEQ ID xx) comprises 488 amino acid residues, has a predicted MW of 55kDa, and a has a putative transit peptide sequence comprising the first 98 amino acids. The predicted MW of the mature form of the *Arabidopsis* homologue is 44kDa. The hydropathic plot for the *Arabidopsis* homologue also reveals that it is hydrophillic (Figure 33). Further blast analysis of the *Arabidopsis* homologue reveals limited sequence identity (25 %

sequence identity) with the beta-subunit of respiratory nitrate reductase. Based on the sequence identity to nitrate reductase, it suggests the slr1737 orf is an enzyme that likely involves general acid catalysis mechanism.

Investigation of known enzymes involved in tocopherol metabolism indicated that the best candidate corresponding to the general acid mechanism is the tocopherol cyclase. There are many known examples of cyclases including, tocopherol cyclase, chalcone isomerase, lycopene cyclase, and aristolochene synthase. By further examination of the microscopic catalytic mechanism of phytoplastoquinol cyclization, as an example, chalcone isomerase has a catalytic mechanism most similar to tocopherol cyclase. (Figure 34).

Multiple sequence alignment was performed between slr1737, slr1737 *Arabidopsis* homologue and the *Arabidopsis* chalcone isomerase (Genbank:P41088) (Figure 35). 65% of the conserved residues among the three enzymes are strictly conserved within the known chalcone isomerases. The crystal structure of alfalfa chalcone isomerase has been solved (Jez, Joseph M., Bowman, Marianne E., Dixon, Richard A., and Noel, Joseph P. (2000) "Structure and mechanism of the evolutionarily unique plant enzyme chalcone isomerase". *Nature Structural Biology* 7: 786-791.) It has been demonstrated tyrosine (Y) 106 of the alfalfa chalcone isomerase serves as the general acid during cyclization reaction (Genbank: P28012). The equivalent residue in slr1737 and the slr1737 *Arabidopsis* homolog is lysine (K), which is an excellent catalytic residue as general acid.

The information available from partial purification of tocopherol cyclase from *Chlorella protothecoides* (U.S. Patent No. 5,432,069), *i.e.*, described as being glycine rich, water soluble and with a predicted MW of 48-50kDa, is consistent with the protein informatics information obtained for the slr1737 and the Arabidopsis slr1737 homologue.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claim.

CLAIMS

What is claimed is:

1. An isolated nucleic acid sequence encoding a tocopherol cyclase.

2. An isolated nucleic acid sequence according to Claim 1, wherein said tocopherol cyclase is active in the cyclization of 2,3-dimethyl-5-phytylplastoquinol to tocopherol.

3. An isolated nucleic acid sequence according to Claim 1, wherein said tocopherol cyclase is active in the cyclization of 2,3-dimethyl-5-geranylgeranylplastoquinol to tocotrienol.

4. An isolated DNA sequence according to Claim 1, wherein said nucleic acid sequence is isolated from a eukaryotic cell source.

5. An isolated DNA sequence according to Claim 4, wherein said eukaryotic cell source is selected from the group consisting of mammalian, nematode, fungal, and plant cells.

6. The DNA encoding sequence of Claim 5 wherein said tocopherol cyclase protein is from *Arabidopsis*.

7. The DNA encoding sequence of Claim 6 wherein said tocopherol cyclase protein is encoded by a sequence of SEQ ID NO:109.

8. The DNA encoding sequence of Claim 7 wherein said tocopherol cyclase protein has an amino acid sequence of SEQ ID NO:110.

9. The DNA encoding sequence of Claim 4 wherein said tocopherol cyclase protein is from a source selected from the group consisting of *Arabidopsis,* soybean, corn, rice, wheat, leek canola, , leek, cotton, and tomato.

10. An isolated DNA sequence according to Claim 4, wherein said prokaryotic source is a *Synechocystis* sp.

11. The DNA encoding sequence of Claim 10 wherein said tocopherol cyclase protein is encoded by a sequence of SEQ ID NO:38.

12. The DNA encoding sequence of Claim 10 wherein said tocopherol cyclase protein has an amino acid sequence of SEQ ID NO:39.

13. A nucleic acid construct comprising as operably linked components, a transcriptional initiation region functional in a host cell, a nucleic acid sequence encoding a tocopherol cyclase, and a transcriptional termination region.

14. A nucleic acid construct according to Claim 13, wherein said nucleic acid sequence encoding tocopherol cyclase is obtained from an organism selected from the group consisting of a eukaryotic organism and a prokaryotic organism.

15. A nucleic acid construct according to Claim 14, wherein said nucleic acid sequence encoding tocopherol cyclase is obtained from a plant source.

16. A nucleic acid construct according to Claim 15, wherein said nucleic acid sequence encoding tocopherol cyclase is obtained from a source selected from the group consisting of *Arabidopsis,* soybean, corn, rice, wheat, leek canola, , leek, cotton, and tomato.

17. A nucleic acid construct according to Claim 13, wherein said nucleic acid sequence encoding tocopherol cyclase is obtained from a *Synechocystis* sp.

18. A plant cell comprising the construct of 13.

19. A plant comprising a cell of Claim 18.

20 A feed composition produced from a plant according to Claim 19.

21. A seed comprising a cell of Claim 18.

22 Oil obtained from a seed of Claim 21.

23. A natural tocopherol rich refined and deodorised oil which has been produced by a method of treating an oil according to Claim 22 by distilling under low pressure and high temperature, wherein said refined oil has reduced free fatty acids and a substantial percentage of tocopherol present in the pretreated oil.

24. A refined oil according to claim 23, wherein the pretreated oil is crude or pre-treated soybean oil.

25. A refined oil according to claim 23, wherein the refined oil is degummed and bleached.

26. A method for the alteration of the isoprenoid content in a host cell, said method comprising; transforming said host cell with a construct comprising as operably linked components, a transcriptional initiation region functional in a host cell, a nucleic acid sequence encoding tocopherol cyclase, and a transcriptional termination region,

wherein said isoprenoid compound selected from the group of tocopherols and tocotrienols .

27. The method according to Claim 26, wherein said host cell is selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

28. The method according to Claim 27, wherein said prokaryotic cell is a *Synechocystis* sp.

29. The method according to Claim 27, wherein said eukaryotic cell is a plant cell.

30. The method according to Claim 29, wherein said plant cell is obtained from a plant selected from the group consisting of *Arabidopsis,* soybean, corn, rice, wheat, leek canola, , leek, cotton, and tomato.

31. A method for producing an isoprenoid compound of interest in a host cell, said method comprising obtaining a transformed host cell, said host cell having and expressing in its genome:

a construct having a DNA sequence encoding a tocopherol cyclase operably linked to a transcriptional initiation region functional in a host cell,

wherein said isoprenoid compound selected from the group of tocopherols and tocotrienols.

32. The method according to Claim 31, wherein said host cell is selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

33. The method according to Claim 32, wherein said prokaryotic cell is a *Synechocystis* sp.

34. The method according to Claim 32, wherein said eukaryotic cell is a plant cell.

35. The method according to Claim 34, wherein said plant cell is obtained from a plant selected from the group consisting wherein said compound selected from the group of *Arabidopsis,* soybean, corn, rice, wheat, leek canola, , leek, cotton, and tomato.

36. A method for increasing the biosynthetic flux in a host cell toward production of an isoprenoid compound, said method comprising;

transforming said host cell with a construct comprising as operably linked components, a transcriptional initiation region functional in a host cell, a DNA encoding a tocopherol cyclase, and a transcriptional termination region,

wherein said isoprenoid compound selected from the group of tocopherols and tocotrienols,.

37. The method according to Claim 36, wherein said host cell is selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

38. The method according to Claim 37, wherein said prokaryotic cell is a *Synechocystis* sp.

39. The method according to Claim 37, wherein said eukaryotic cell is a plant cell.

40. The method according to Claim 39, wherein said plant cell is obtained from a plant selected from the group consisting *Arabidopsis,* soybean, corn, rice, wheat, leek canola, , leek, cotton, and tomato.

41. The method according to Claim 39, wherein said transcriptional initiation region is a seed-specific promoter.

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NUCLEIC ACID SEQUENCES TO PROTEINS INVOLVED IN TOCOPHEROL SYNTHESIS

ABSTRACT

Nucleic acid sequences and methods are provided for producing plants and seeds having altered tocopherol content and compositions. The methods find particular use in increasing the tocopherol levels in plants, and in providing desirable tocopherol compositions in a host plant cell.

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                         *         20          *         40          *         60          *         80          *
ATPT2:  -------ME---LSSSSLVSAAGG------FCWKKQN----LKLHSLEIRVLRCDSKVAKPK------FR----NNLRP--DGQG :  59
ATPT3:  MAFFGLSRVRRLKSSVSVTPSSSALLQSQHKSLSNPVTTHYTNPFKCYPSWNDNYQWSKGRELHQEKFFGVGWNYICGMSSSS :  90
ATPT4:  -----MWRRS-VYRFSSRISVSSSLPNPRLIPWSREL----CAVNSFQPPVSTESAKGITGV-----RSD----ANFA--TATA :  69
ATPT8:  --------------MVLAEVPKLAS-----AAEYFFKR------GVQGKQFR----SILLMATA------LN-----VPE--ALIG :  48
ATPT12: -------MT---LNTVSTIHSSRVTSVDRVGVLSLRN----SDSVEFRRR-SGFSLIYESPGR-----RFV-----VAAE--TDTD :  64
               s         6                                                                     r

           100         *       120         *       140         *       160         *       180
ATPT2  : SSLLLYP-----KHKSRFRNATAGQPEAFDSNSKQKSFRDSDAFYRFSR---PHTVIGTVLSILSVSLAVEKVSDISPLLFTGILEA : 141
ATPT3  : SVLEGKPKKDDKEKSDGVVKKASWIDLYLPEEVRGYAKLARDKPIGTWLLAWPCMWSIAL-AADPGSLPSF--K------YMALFGG : 171
ATPT4  : AATATAT----TG-EISSRAALAGLGHHYARCYWELSK-AKSMLYVATS---GTGYLGT-GNAAISPGL----------C--YTA : 137
ATPT8  : ESTDIVT----SELRVRQRGIAEITEMIHVASLLHDDVL-DDADTRRGVGS---LNVVMGNKMSVLAGDLLS-------------RAG : 117
ATPT12 : KVKSQTP---DKAPAGGSSNQLLGIKG-ASQETNKWKIRLQTKPV-TWP---FLVWGVVCGAAASGNHWTPED------VAKSILM : 140
                                                      l                     f                 c

               *       200         *       220         *       240         *       260         *
ATPT2  : VAALMMNIYIVGLQLSDVEIKVNKP--YLIASEYVNTGAIASFSIMS-FWGWIVGSWPLFWA----LFVSFMLGTAYSINL : 224
ATPT3  : A---LLRGAGCTDLLDQDIТKVDRTKLRIASLLPFQGGFGLQLLLG-LGLLQLNYS--------RVLGASSLLLVESY : 248
ATPT4  : GT--MMIAASANSQIFEISNSKMKRTMLRLPSRIVPHAAWATIAGASGACLASKTMLAAG--------LASANLVLYAEVYTP : 219
ATPT8  : A---AALKNTEVVALLATAVEHLVTGET---METTSSTEQRYSMDYYQKTYYKT---ASLISSCK---------AVAVLTGQTAEVAV : 190
ATPT12 : MSGPCLTGYTQTDWYDRDIAINEP--YRIPSAIEPEVTQWVLLLGG-LGACILVWAGHTTPTVFYLALGGSLLSYTYSA : 227
               6        n        d             p6 Sg

             280         *       300         *       320         *       340         *       360
ATPT2  : PLLRWKRFALAAMCILAVRAIIVQLAFLHIQTHVFGRPILFTRPLIFATAFMSFFS-VIALFKDIPDIG--------D------KI : 299
ATPT3  : P-LMKRFTFWPQAFLGLT--INWGALGT-----VKGSIAPSILP---LYLSGVCWTVYDTIYAHQDKD---------D------VK : 314
ATPT4  : LKQLHPINTWGAVV-----GAIPPLGA-----ASGQESYNSTLPAALYFWQIPHFAIAHLCRNDYAAGGYKMLSLFDPSGKRIAA : 300
ATPT8  : LAFEYGRNLGAFQLI----DDILDFTGTS-----SLGKGSLSDRHGVITAPILFAMEEFPQLREVVDQVK--------DP-----RN : 259
ATPT12 : PPLKLKQNGWGNFA-LG--ASYISPWAGQ---LFGTLTPDVVL---TLLYSIAG-GIAIVNDFKSVG--------D------RA : 294
                                            a G                                    e          D

               *       380         *       400         *       420         *       440         *
ATPT2  : FGIRS----FSVTLGQ----KRVFTC--VTLLQMAYAVAILVGATSPFIWSK----VISVGHVILATTLWARAKSVDLSSKTEITSCY : 375
ATPT3  : GVKS----TALRFGD----NTKLLTGFGTASIGFALSGESADLGWQYYAS----LAAASGQLGWQIGTADLSSGADCSRKFVSNKWE : 392
ATPT4  : ALRNCFYMIPLGFIAYDWGLTSSFCLESTLLTIAAATAFSFYRDRTMHKARKMFHASLFLPVFMSGLLLHRVSNDNQQQLVEEAGL : 390
ATPT8  : DIAL----EYLGKSK--------GIQ---RARELAEHANLAAAAIGSLPET----DNEDKRSRRALIDLTHRVITRNK--------- : 321
ATPT12 : GLQS----LPVAEGT----ETAKTG-VGAIDITQSVAGYLLASGKPYYALA--LVALIPQTVEQFKYFLKDPVKYDVKYQASAQPF : 373
           6       6               w

              460         *       480         *
ATPT2  : -MFWKLFYAE----YLLLPFLK------------------ : 393
ATPT3  : GAIFSGVVLG----RSFQ---------------------- : 407
ATPT4  : TNSSGEVKTQRRKKRVAQPPVAYASAAPFPFLPAPSFYSP : 431
ATPT8  : ---------------------------------------- :   -
ATPT12 : --LLGIFVTA----LASQH--------------------- : 387
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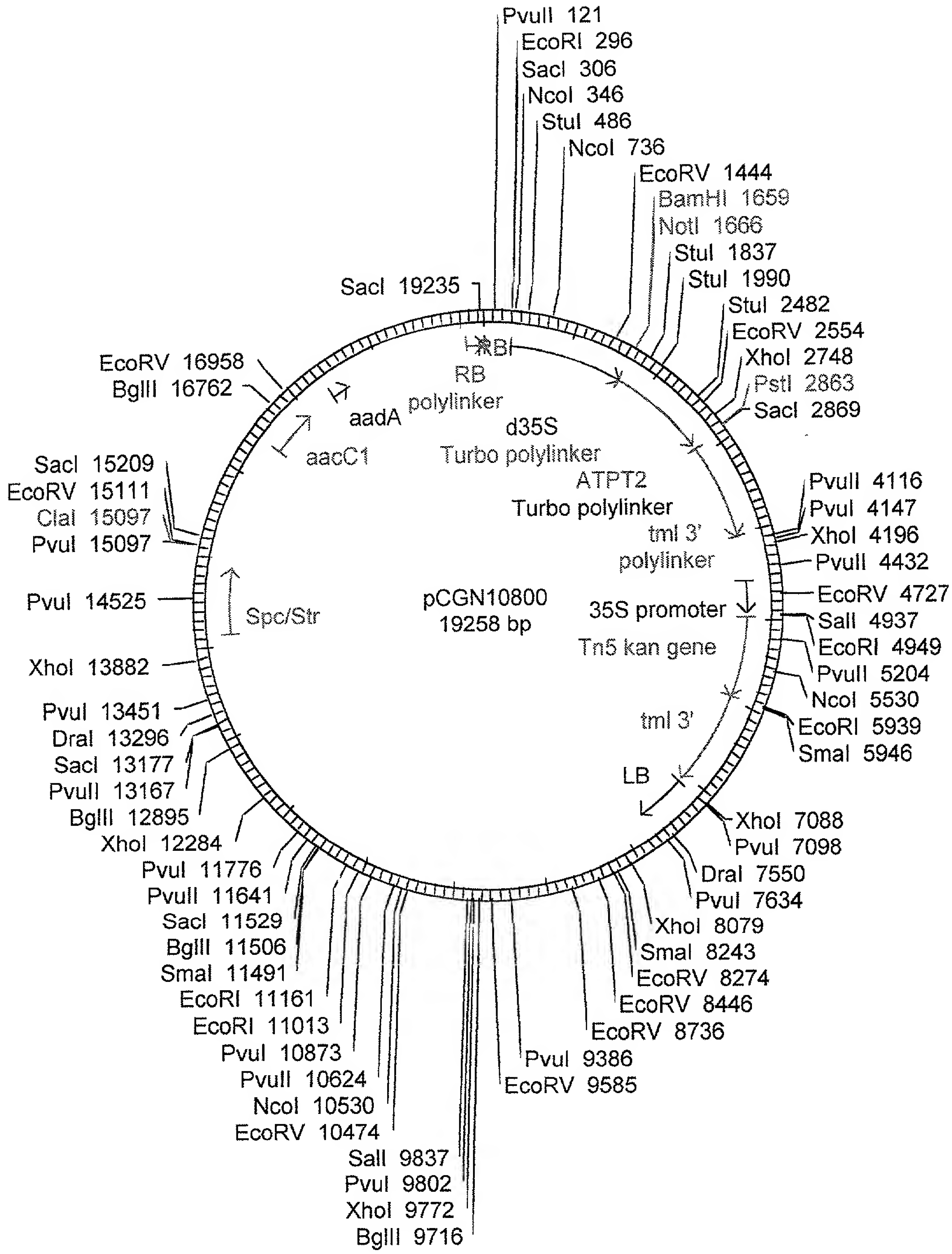
Figure 1

09588059.101400



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SacI 306
NcoI 346
StuI 486
NcoI 736
EcoRV 1444
BamHI 1659
NotI 1666
StuI 1837
StuI 1990
StuI 2482
EcoRV 2554
XhoI 2748
PstI 2863
SacI 2869
PvuII 4116
PvuI 4147
XhoI 4196
PvuII 4432
EcoRV 4727
SalI 4937
EcoRI 4949
PvuII 5204
NcoI 5530
EcoRI 5939
SmaI 5946
XhoI 7088
PvuI 7098
DraI 7550
PvuI 7634
XhoI 8079
SmaI 8243
EcoRV 8274
EcoRV 8446
EcoRV 8736
PvuI 9386
EcoRV 9585
SalI 9837
PvuI 9802
XhoI 9772
BglII 9716
EcoRV 10474
NcoI 10530
PvuII 10624
PvuI 10873
EcoRI 11013
EcoRI 11161
SmaI 11491
BglII 11506
SacI 11529
PvuII 11641
PvuI 11776
XhoI 12284
BglII 12895
PvuII 13167
SacI 13177
DraI 13296
PvuI 13451
XhoI 13882
PvuI 14525
PvuI 15097
ClaI 15097
EcoRV 15111
SacI 15209
BglII 16762
EcoRV 16958
SacI 19235
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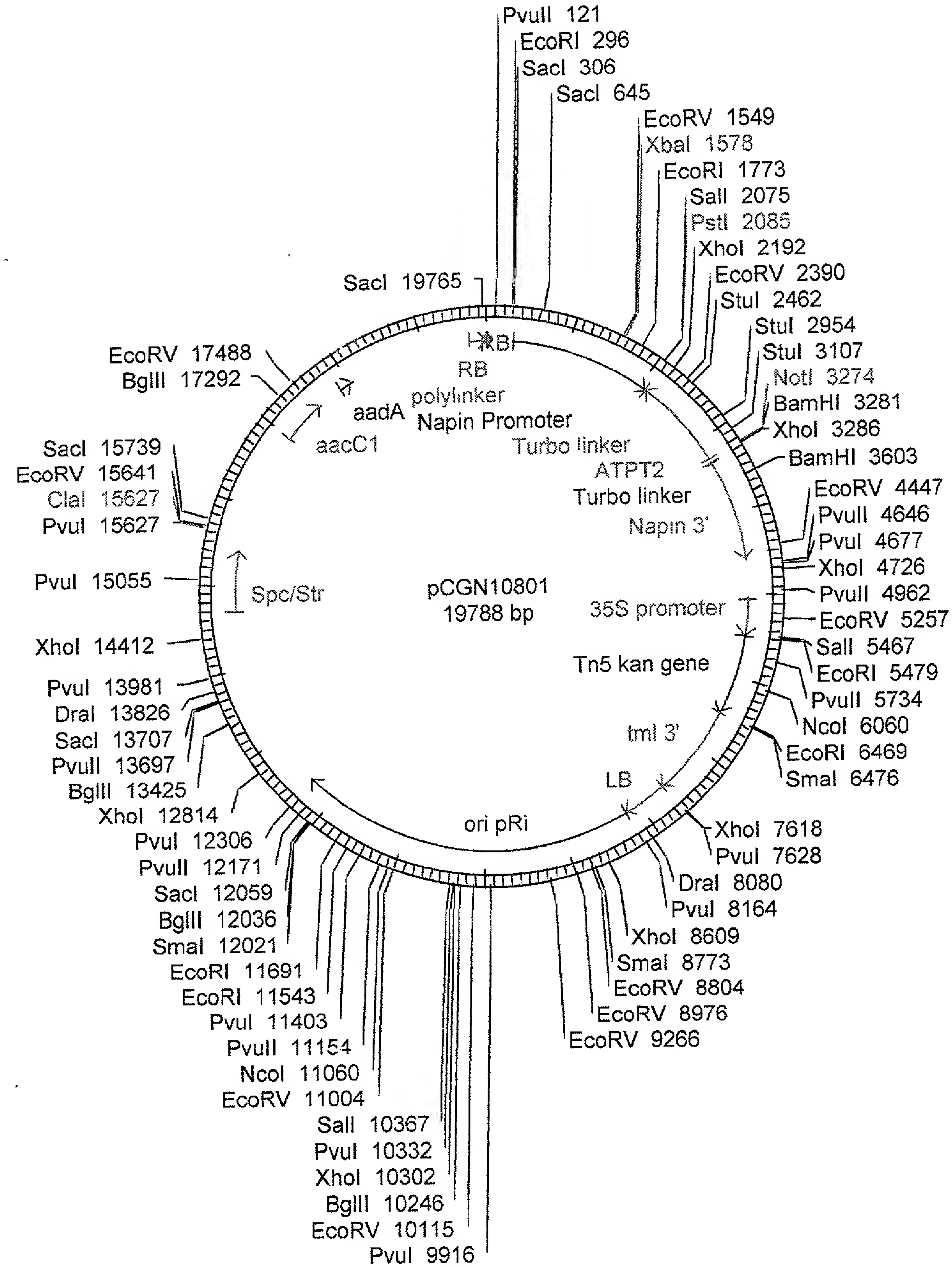
RB
polylinker
aadA
aacC1
d35S
Turbo polylinker
ATPT2
Turbo polylinker
tml 3'
polylinker
35S promoter
Tn5 kan gene
tml 3'
LB
Spc/Str
pCGN10800
19258 bp

Figure 2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SacI 645
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XbaI 1578
EcoRI 1773
SalI 2075
PstI 2085
XhoI 2192
EcoRV 2390
StuI 2462
StuI 2954
StuI 3107
NotI 3274
BamHI 3281
XhoI 3286
BamHI 3603
EcoRV 4447
PvuII 4646
PvuI 4677
XhoI 4726
PvuII 4962
EcoRV 5257
SalI 5467
EcoRI 5479
PvuII 5734
NcoI 6060
EcoRI 6469
SmaI 6476
XhoI 7618
PvuI 7628
DraI 8080
PvuI 8164
XhoI 8609
SmaI 8773
EcoRV 8804
EcoRV 8976
EcoRV 9266
PvuI 9916
EcoRV 10115
BglII 10246
XhoI 10302
PvuI 10332
SalI 10367
EcoRV 11004
NcoI 11060
PvuII 11154
PvuI 11403
EcoRI 11543
EcoRI 11691
SmaI 12021
BglII 12036
SacI 12059
PvuII 12171
PvuI 12306
XhoI 12814
BglII 13425
PvuII 13697
SacI 13707
DraI 13826
PvuI 13981
XhoI 14412
PvuI 15055
PvuI 15627
ClaI 15627
EcoRV 15641
SacI 15739
BglII 17292
EcoRV 17488
SacI 19765
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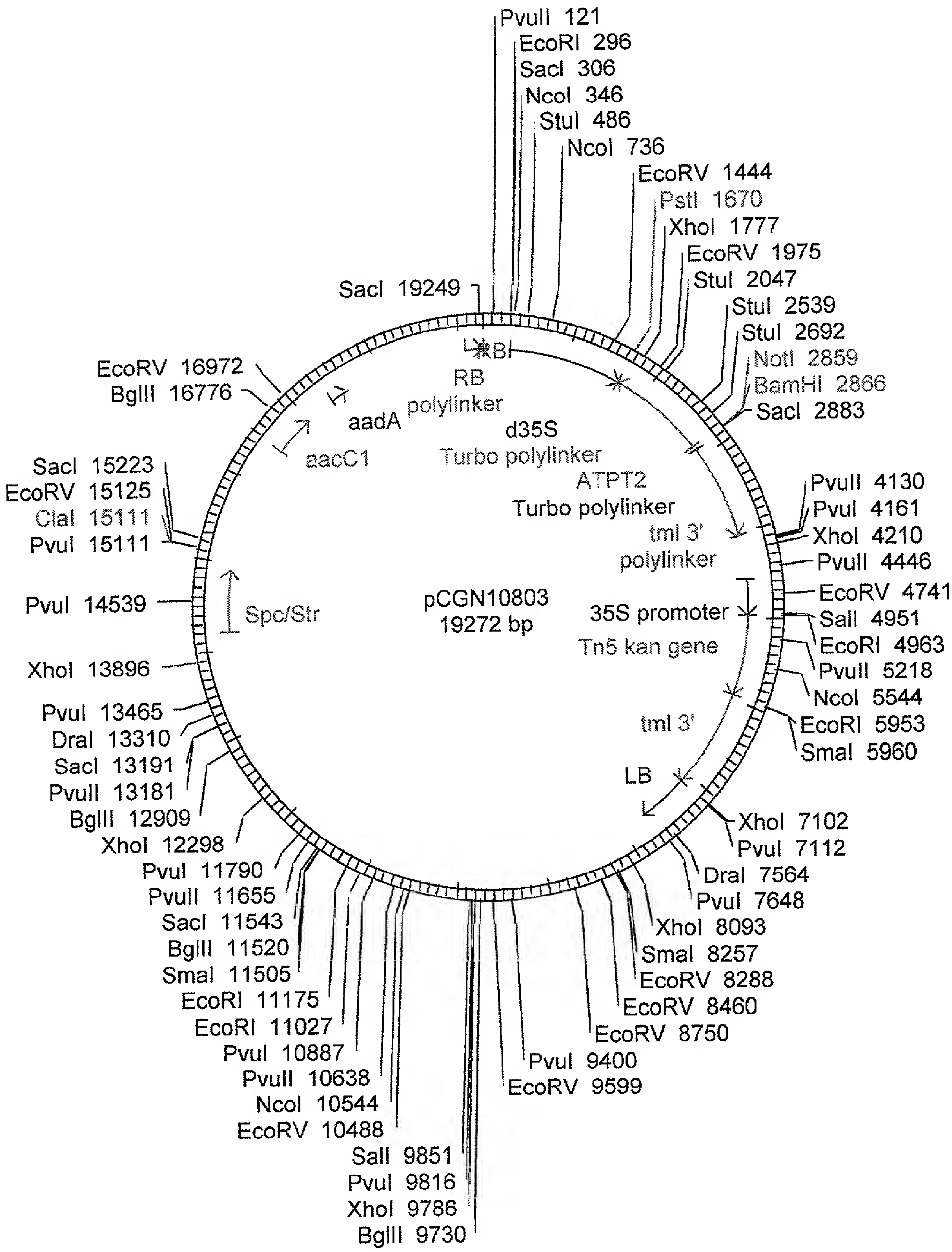
RB
polylinker
Napin Promoter
Turbo linker
ATPT2
Turbo linker
Napin 3'
35S promoter
Tn5 kan gene
tml 3'
LB
ori pRi
Spc/Str
aacC1
aadA
pCGN10801
19788 bp

Figure 3



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NcoI 346
StuI 486
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XhoI 1777
EcoRV 1975
StuI 2047
StuI 2539
StuI 2692
NotI 2859
BamHI 2866
SacI 2883
PvuII 4130
PvuI 4161
XhoI 4210
PvuII 4446
EcoRV 4741
SalI 4951
EcoRI 4963
PvuII 5218
NcoI 5544
EcoRI 5953
SmaI 5960
XhoI 7102
PvuI 7112
DraI 7564
PvuI 7648
XhoI 8093
SmaI 8257
EcoRV 8288
EcoRV 8460
EcoRV 8750
PvuI 9400
EcoRV 9599
SalI 9851
PvuI 9816
XhoI 9786
BglII 9730
EcoRV 10488
NcoI 10544
PvuII 10638
PvuI 10887
EcoRI 11027
EcoRI 11175
SmaI 11505
BglII 11520
SacI 11543
PvuII 11655
PvuI 11790
XhoI 12298
BglII 12909
PvuII 13181
SacI 13191
DraI 13310
PvuI 13465
XhoI 13896
PvuI 14539
PvuI 15111
ClaI 15111
EcoRV 15125
SacI 15223
BglII 16776
EcoRV 16972
SacI 19249
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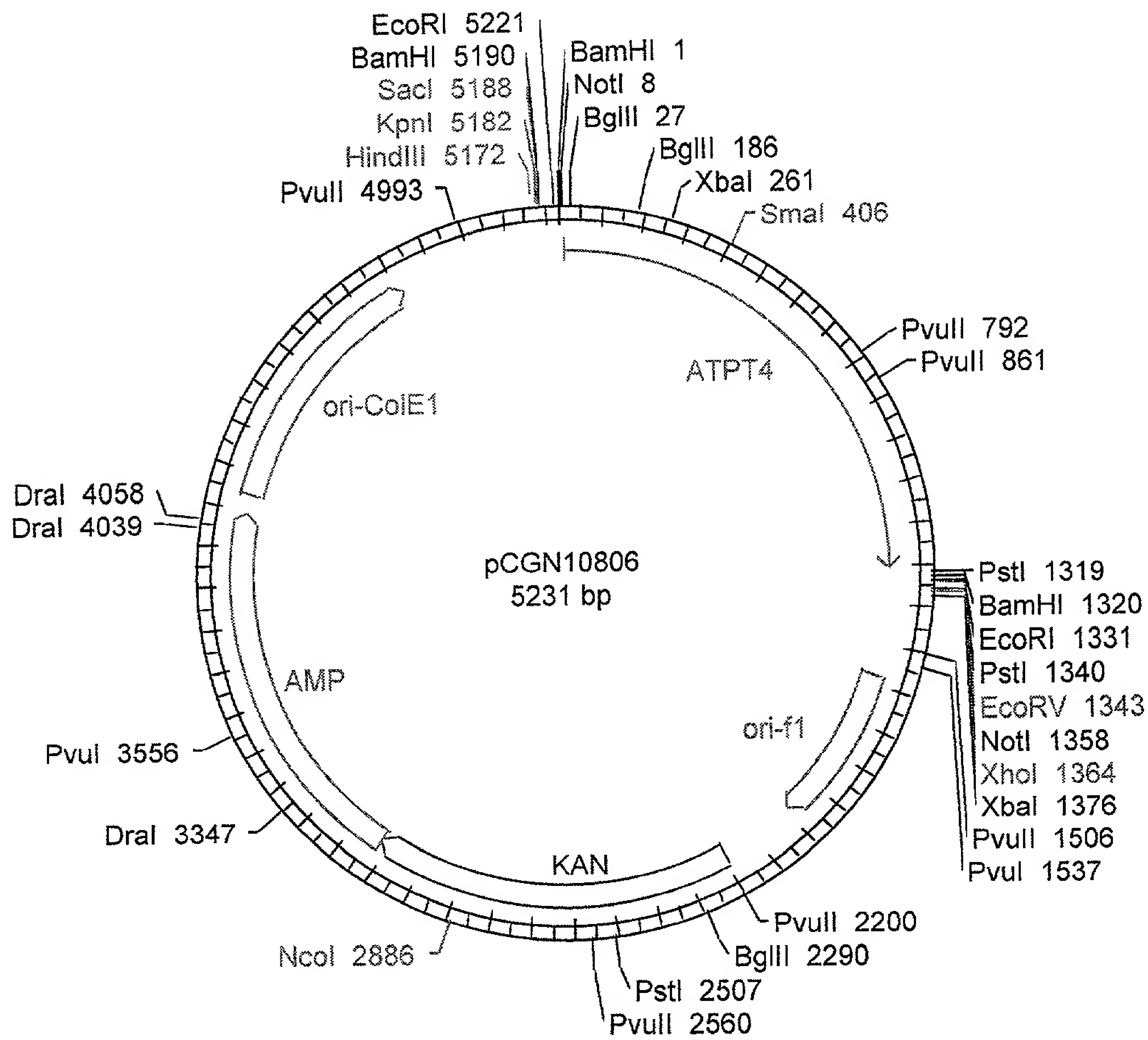
BI
polylinker
d35S
Turbo polylinker
ATPT2
Turbo polylinker
tml 3'
polylinker
35S promoter
Tn5 kan gene
tml 3'
LB
Spc/Str
aacC1
aadA

Figure 4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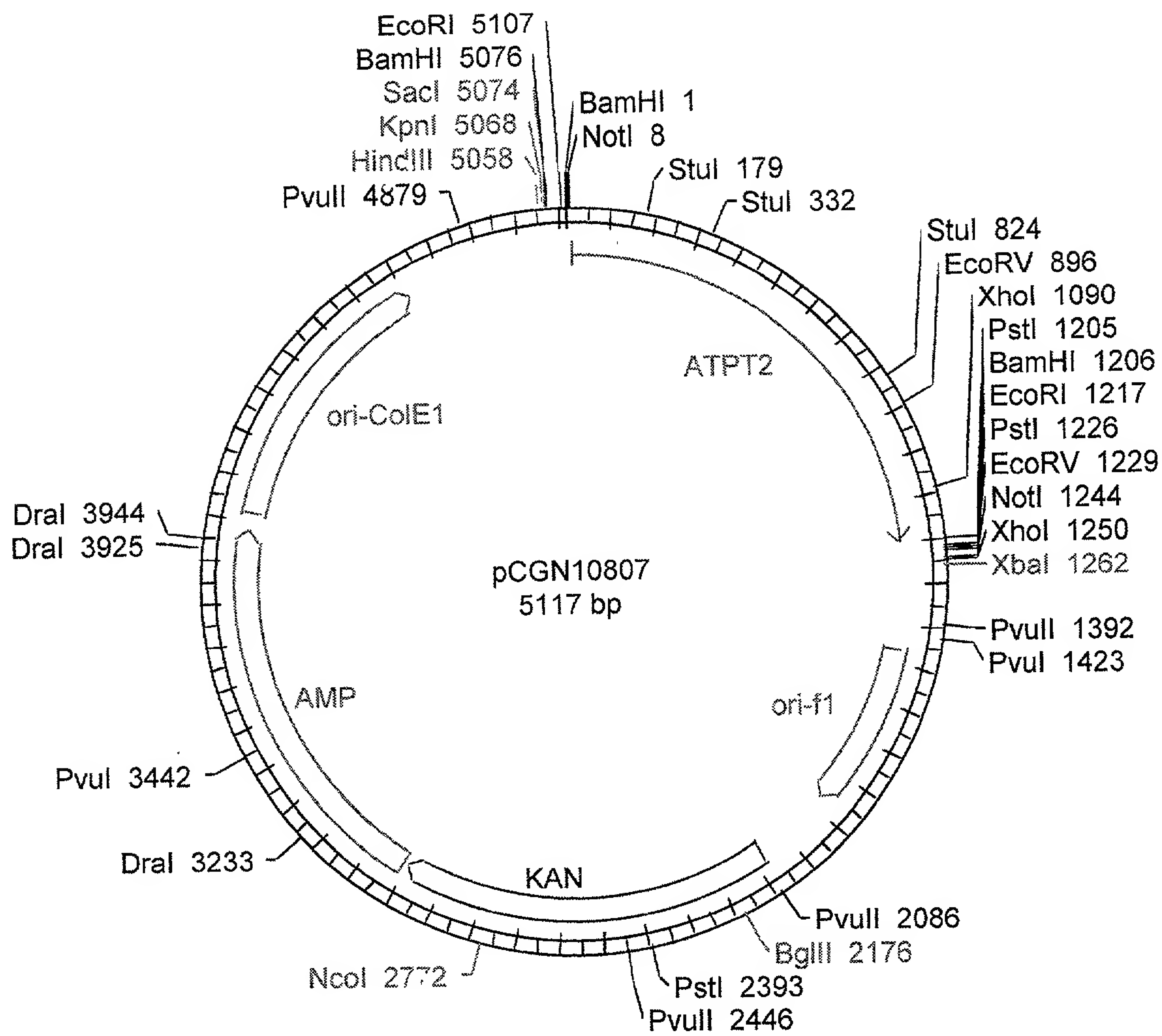
BamHI 5190
SacI 5188
KpnI 5182
HindIII 5172
PvuII 4993
BamHI 1
NotI 8
BglII 27
BglII 186
XbaI 261
SmaI 406
PvuII 792
PvuII 861
ATPT4
ori-ColE1
DraI 4058
DraI 4039
pCGN10806
5231 bp
PstI 1319
BamHI 1320
EcoRI 1331
PstI 1340
EcoRV 1343
NotI 1358
XhoI 1364
XbaI 1376
PvuII 1506
PvuI 1537
AMP
ori-f1
PvuI 3556
DraI 3347
KAN
PvuII 2200
BglII 2290
NcoI 2886
PstI 2507
PvuII 2560

Figure 5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BamHI 5076
SacI 5074
KpnI 5068
HindIII 5058
PvuII 4879
BamHI 1
NotI 8
StuI 179
StuI 332
StuI 824
EcoRV 896
XhoI 1090
PstI 1205
BamHI 1206
EcoRI 1217
PstI 1226
EcoRV 1229
NotI 1244
XhoI 1250
XbaI 1262
PvuII 1392
PvuI 1423
PvuII 2086
BglII 2176
PstI 2393
PvuII 2446
NcoI 2772
DraI 3233
PvuI 3442
DraI 3925
DraI 3944
ATPT2
ori-ColE1
pCGN10807
5117 bp
AMP
ori-f1
KAN

Figure 6

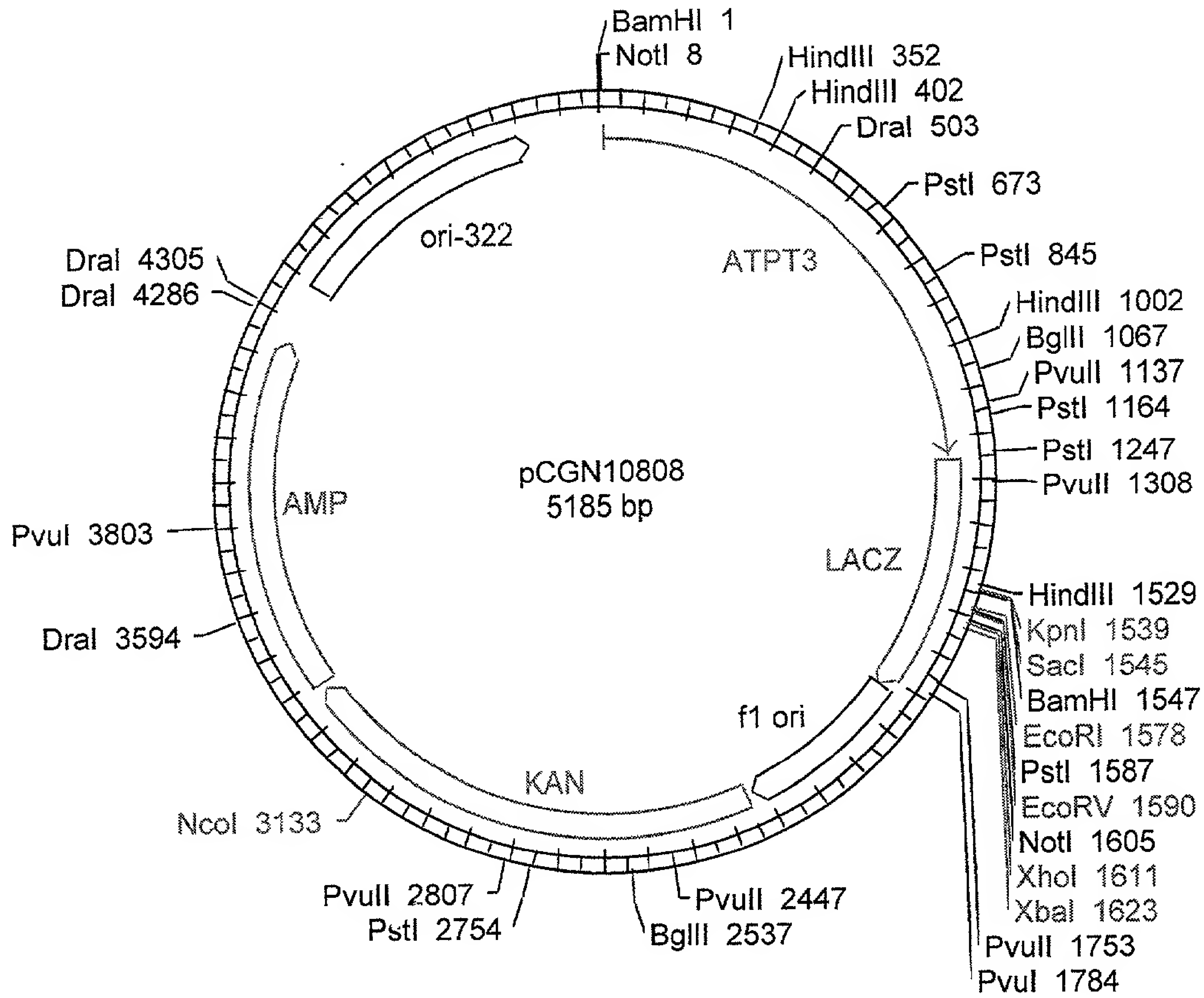
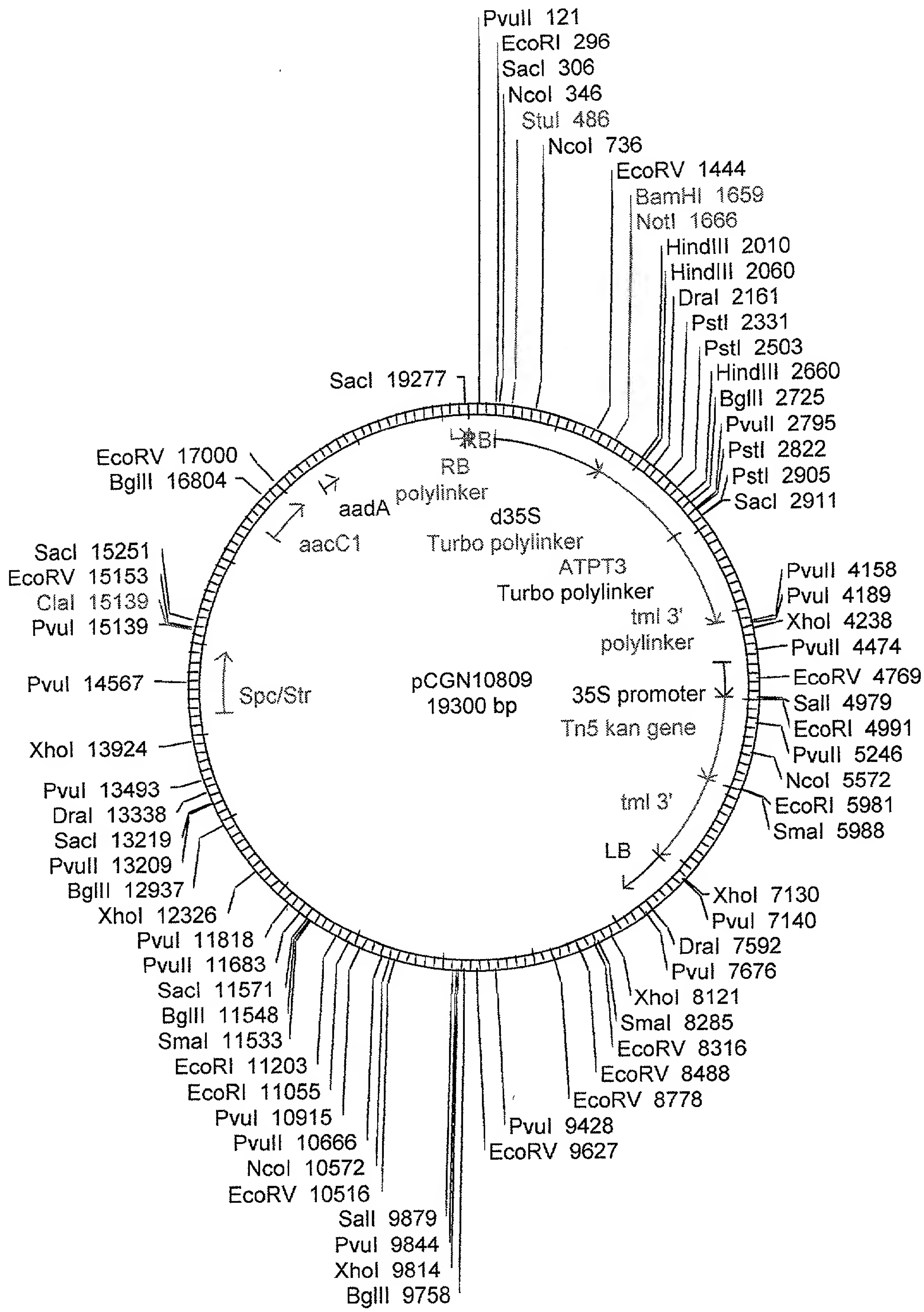

BamHI 1
NotI 8
HindIII 352
HindIII 402
DraI 503
PstI 673
PstI 845
HindIII 1002
BglII 1067
PvuII 1137
PstI 1164
PstI 1247
PvuII 1308
HindIII 1529
KpnI 1539
SacI 1545
BamHI 1547
EcoRI 1578
PstI 1587
EcoRV 1590
NotI 1605
XhoI 1611
XbaI 1623
PvuII 1753
PvuI 1784
PvuII 2447
BglII 2537
PstI 2754
PvuII 2807
NcoI 3133
DraI 3594
PvuI 3803
DraI 4286
DraI 4305
ori-322
ATPT3
AMP
pCGN10808
5185 bp
LACZ
f1 ori
KAN

Figure 7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SacI 306
NcoI 346
StuI 486
NcoI 736
EcoRV 1444
BamHI 1659
NotI 1666
HindIII 2010
HindIII 2060
DraI 2161
PstI 2331
PstI 2503
HindIII 2660
BglII 2725
PvuII 2795
PstI 2822
PstI 2905
SacI 2911
PvuII 4158
PvuI 4189
XhoI 4238
PvuII 4474
EcoRV 4769
SalI 4979
EcoRI 4991
PvuII 5246
NcoI 5572
EcoRI 5981
SmaI 5988
XhoI 7130
PvuI 7140
DraI 7592
PvuI 7676
XhoI 8121
SmaI 8285
EcoRV 8316
EcoRV 8488
EcoRV 8778
PvuI 9428
EcoRV 9627
BglII 9758
XhoI 9814
PvuI 9844
SalI 9879
EcoRV 10516
NcoI 10572
PvuII 10666
PvuI 10915
EcoRI 11055
EcoRI 11203
SmaI 11533
BglII 11548
SacI 11571
PvuII 11683
PvuI 11818
XhoI 12326
BglII 12937
PvuII 13209
SacI 13219
DraI 13338
PvuI 13493
XhoI 13924
PvuI 14567
PvuI 15139
ClaI 15139
EcoRV 15153
SacI 15251
BglII 16804
EcoRV 17000
SacI 19277
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polylinker
d35S
Turbo polylinker
ATPT3
Turbo polylinker
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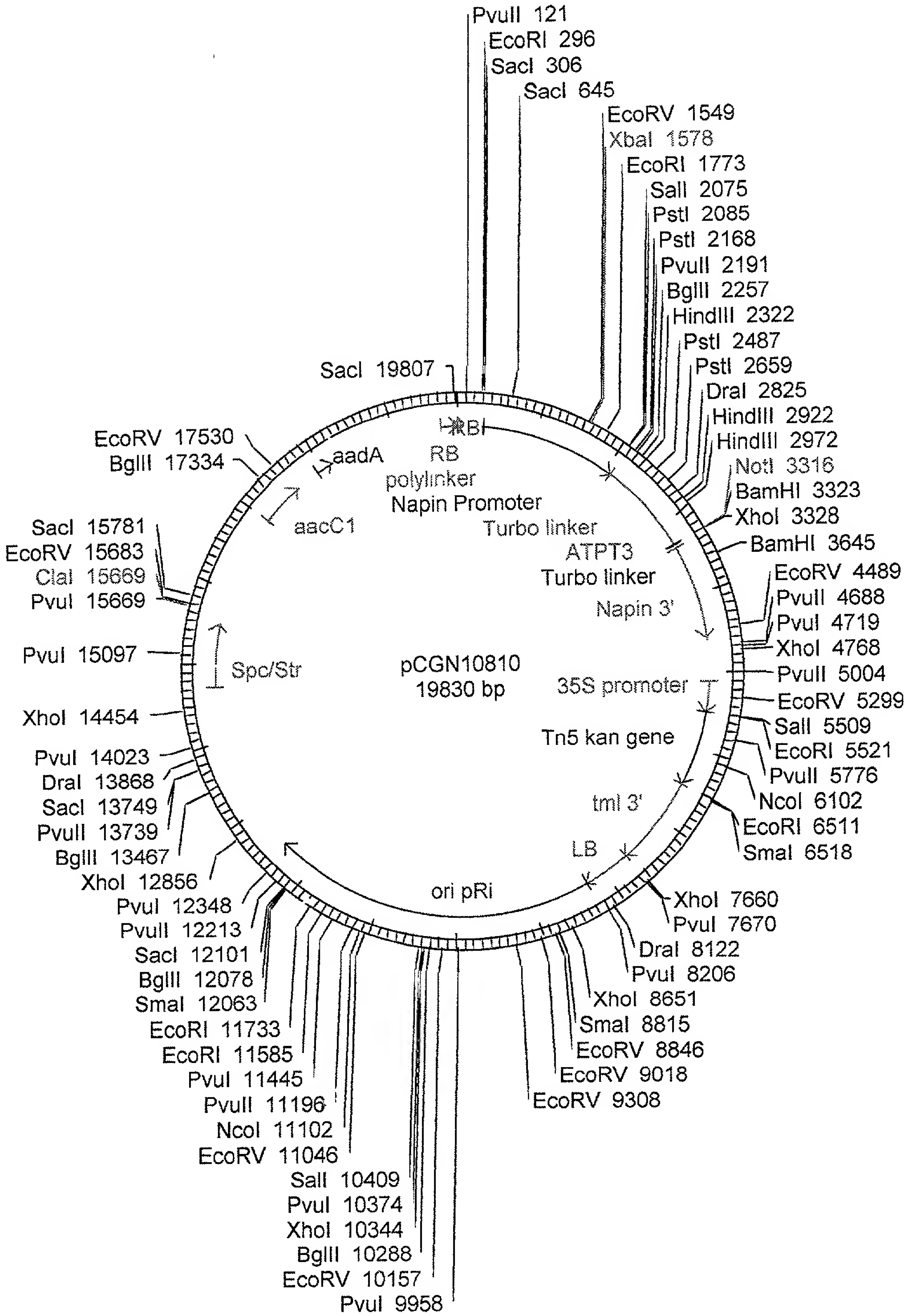
polylinker
35S promoter
Tn5 kan gene
tml 3'
LB
aadA
aacC1
Spc/Str
pCGN10809
19300 bp

Figure 8



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XbaI 1578
EcoRI 1773
SalI 2075
PstI 2085
PstI 2168
PvuII 2191
BglII 2257
HindIII 2322
PstI 2487
PstI 2659
DraI 2825
HindIII 2922
HindIII 2972
NotI 3316
BamHI 3323
XhoI 3328
BamHI 3645
EcoRV 4489
PvuII 4688
PvuI 4719
XhoI 4768
PvuII 5004
EcoRV 5299
SalI 5509
EcoRI 5521
PvuII 5776
NcoI 6102
EcoRI 6511
SmaI 6518
XhoI 7660
PvuI 7670
DraI 8122
PvuI 8206
XhoI 8651
SmaI 8815
EcoRV 8846
EcoRV 9018
EcoRV 9308
PvuI 9958
EcoRV 10157
BglII 10288
XhoI 10344
PvuI 10374
SalI 10409
EcoRV 11046
NcoI 11102
PvuII 11196
PvuI 11445
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EcoRI 11733
SmaI 12063
BglII 12078
SacI 12101
PvuII 12213
PvuI 12348
XhoI 12856
BglII 13467
PvuII 13739
SacI 13749
DraI 13868
PvuI 14023
XhoI 14454
PvuI 15097
PvuI 15669
ClaI 15669
EcoRV 15683
SacI 15781
BglII 17334
EcoRV 17530
SacI 19807
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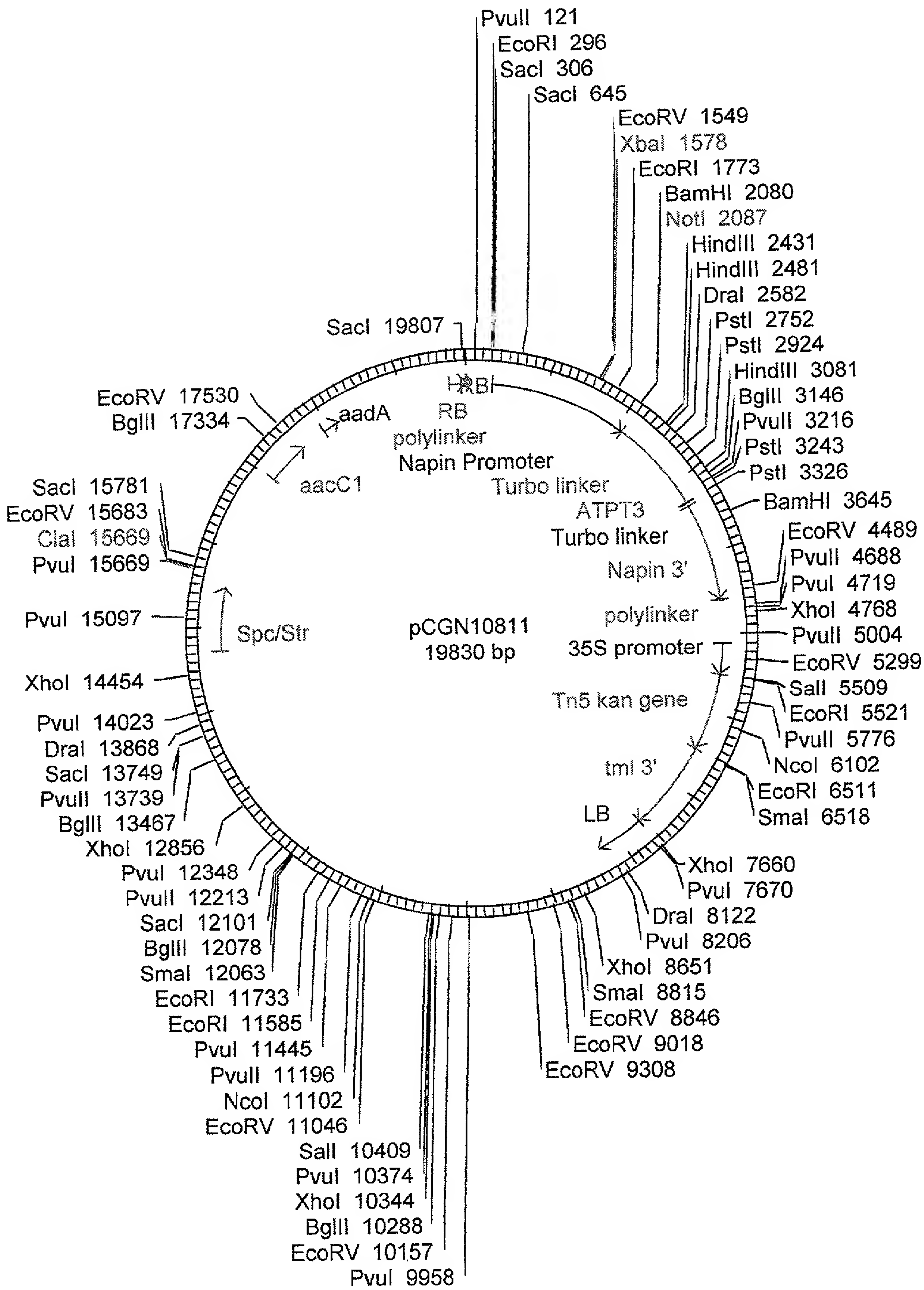
aadA
RB
polylinker
Napin Promoter
aacC1
Turbo linker
ATPT3
Turbo linker
Napin 3'
Spc/Str
pCGN10810
19830 bp
35S promoter
Tn5 kan gene
tml 3'
LB
ori pRi

Figure 9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SacI 306
SacI 645
EcoRV 1549
XbaI 1578
EcoRI 1773
BamHI 2080
NotI 2087
HindIII 2431
HindIII 2481
DraI 2582
PstI 2752
PstI 2924
HindIII 3081
BglII 3146
PvuII 3216
PstI 3243
PstI 3326
BamHI 3645
EcoRV 4489
PvuII 4688
PvuI 4719
XhoI 4768
PvuII 5004
EcoRV 5299
SalI 5509
EcoRI 5521
PvuII 5776
NcoI 6102
EcoRI 6511
SmaI 6518
XhoI 7660
PvuI 7670
DraI 8122
PvuI 8206
XhoI 8651
SmaI 8815
EcoRV 8846
EcoRV 9018
EcoRV 9308
PvuI 9958
EcoRV 10157
BglII 10288
XhoI 10344
PvuI 10374
SalI 10409
EcoRV 11046
NcoI 11102
PvuII 11196
PvuI 11445
EcoRI 11585
EcoRI 11733
SmaI 12063
BglII 12078
SacI 12101
PvuII 12213
PvuI 12348
XhoI 12856
BglII 13467
PvuII 13739
SacI 13749
DraI 13868
PvuI 14023
XhoI 14454
PvuI 15097
PvuI 15669
ClaI 15669
EcoRV 15683
SacI 15781
BglII 17334
EcoRV 17530
SacI 19807
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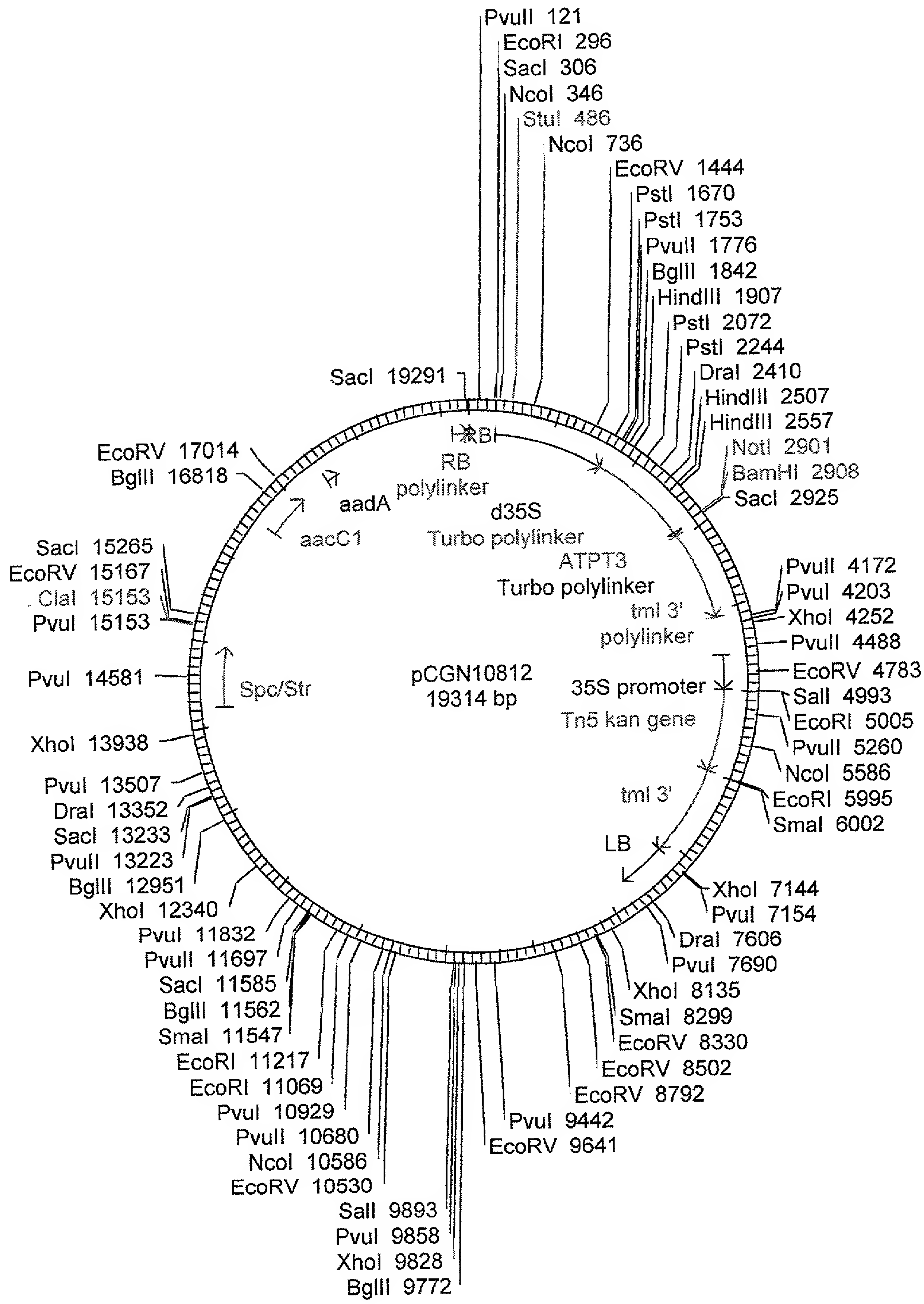
polylinker
Napin Promoter
aacC1
Turbo linker
ATPT3
Turbo linker
Napin 3'
polylinker
35S promoter
Tn5 kan gene
tml 3'
LB
Spc/Str
pCGN10811
19830 bp

Figure 10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NcoI 346
StuI 486
NcoI 736
EcoRV 1444
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PstI 1753
PvuII 1776
BglII 1842
HindIII 1907
PstI 2072
PstI 2244
DraI 2410
HindIII 2507
HindIII 2557
NotI 2901
BamHI 2908
SacI 2925
PvuII 4172
PvuI 4203
XhoI 4252
PvuII 4488
EcoRV 4783
SalI 4993
EcoRI 5005
PvuII 5260
NcoI 5586
EcoRI 5995
SmaI 6002
XhoI 7144
PvuI 7154
DraI 7606
PvuI 7690
XhoI 8135
SmaI 8299
EcoRV 8330
EcoRV 8502
EcoRV 8792
PvuI 9442
EcoRV 9641
BglII 9772
XhoI 9828
PvuI 9858
SalI 9893
EcoRV 10530
NcoI 10586
PvuII 10680
PvuI 10929
EcoRI 11069
EcoRI 11217
SmaI 11547
BglII 11562
SacI 11585
PvuII 11697
PvuI 11832
XhoI 12340
BglII 12951
PvuII 13223
SacI 13233
DraI 13352
PvuI 13507
XhoI 13938
PvuI 14581
PvuI 15153
ClaI 15153
EcoRV 15167
SacI 15265
BglII 16818
EcoRV 17014
SacI 19291
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RB
polylinker
d35S
Turbo polylinker
ATPT3
Turbo polylinker
tml 3'
polylinker
35S promoter
Tn5 kan gene
tml 3'
LB
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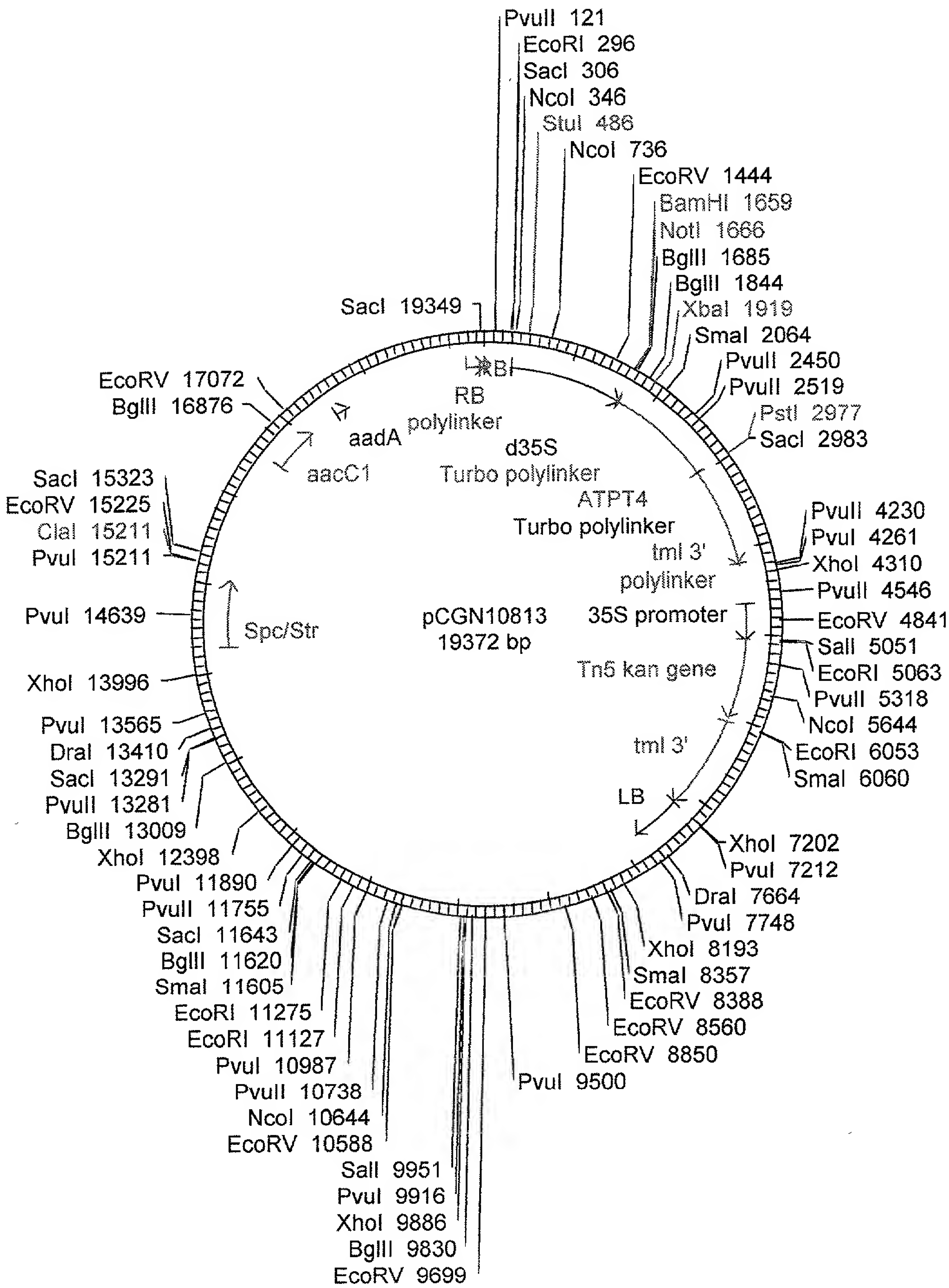
aacC1
Spc/Str
pCGN10812
19314 bp

Figure 11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SacI 306
NcoI 346
StuI 486
NcoI 736
EcoRV 1444
BamHI 1659
NotI 1666
BglII 1685
BglII 1844
XbaI 1919
SmaI 2064
PvuII 2450
PvuII 2519
PstI 2977
SacI 2983
PvuII 4230
PvuI 4261
XhoI 4310
PvuII 4546
EcoRV 4841
SalI 5051
EcoRI 5063
PvuII 5318
NcoI 5644
EcoRI 6053
SmaI 6060
XhoI 7202
PvuI 7212
DraI 7664
PvuI 7748
XhoI 8193
SmaI 8357
EcoRV 8388
EcoRV 8560
EcoRV 8850
PvuI 9500
EcoRV 9699
BglII 9830
XhoI 9886
PvuI 9916
SalI 9951
EcoRV 10588
NcoI 10644
PvuII 10738
PvuI 10987
EcoRI 11127
EcoRI 11275
SmaI 11605
BglII 11620
SacI 11643
PvuII 11755
PvuI 11890
XhoI 12398
BglII 13009
PvuII 13281
SacI 13291
DraI 13410
PvuI 13565
XhoI 13996
PvuI 14639
PvuI 15211
ClaI 15211
EcoRV 15225
SacI 15323
BglII 16876
EcoRV 17072
SacI 19349
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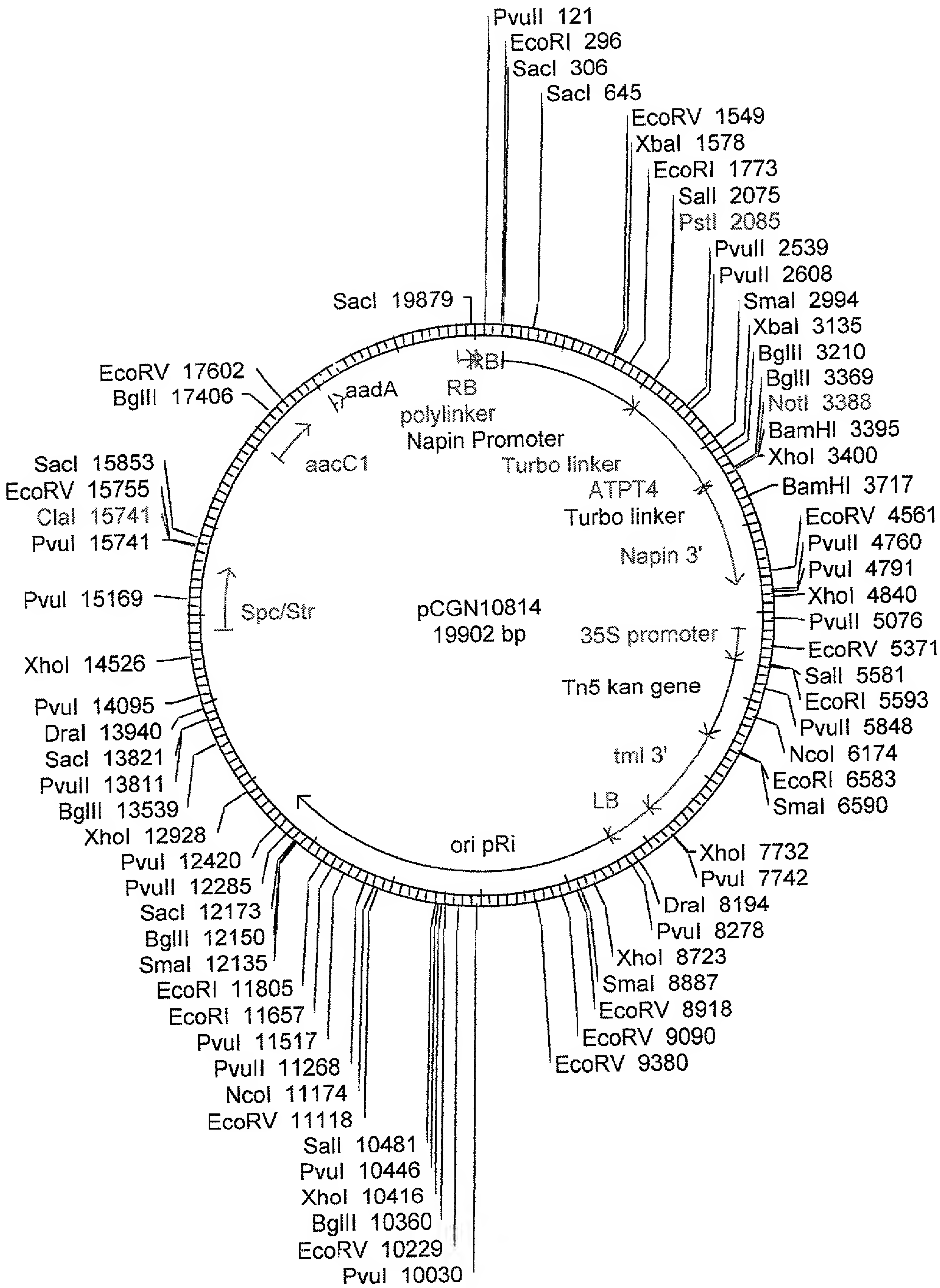
d35S
Turbo polylinker
ATPT4
Turbo polylinker
tml 3'
polylinker
35S promoter
Tn5 kan gene
tml 3'
LB
aadA
aacC1
Spc/Str
pCGN10813
19372 bp

Figure 12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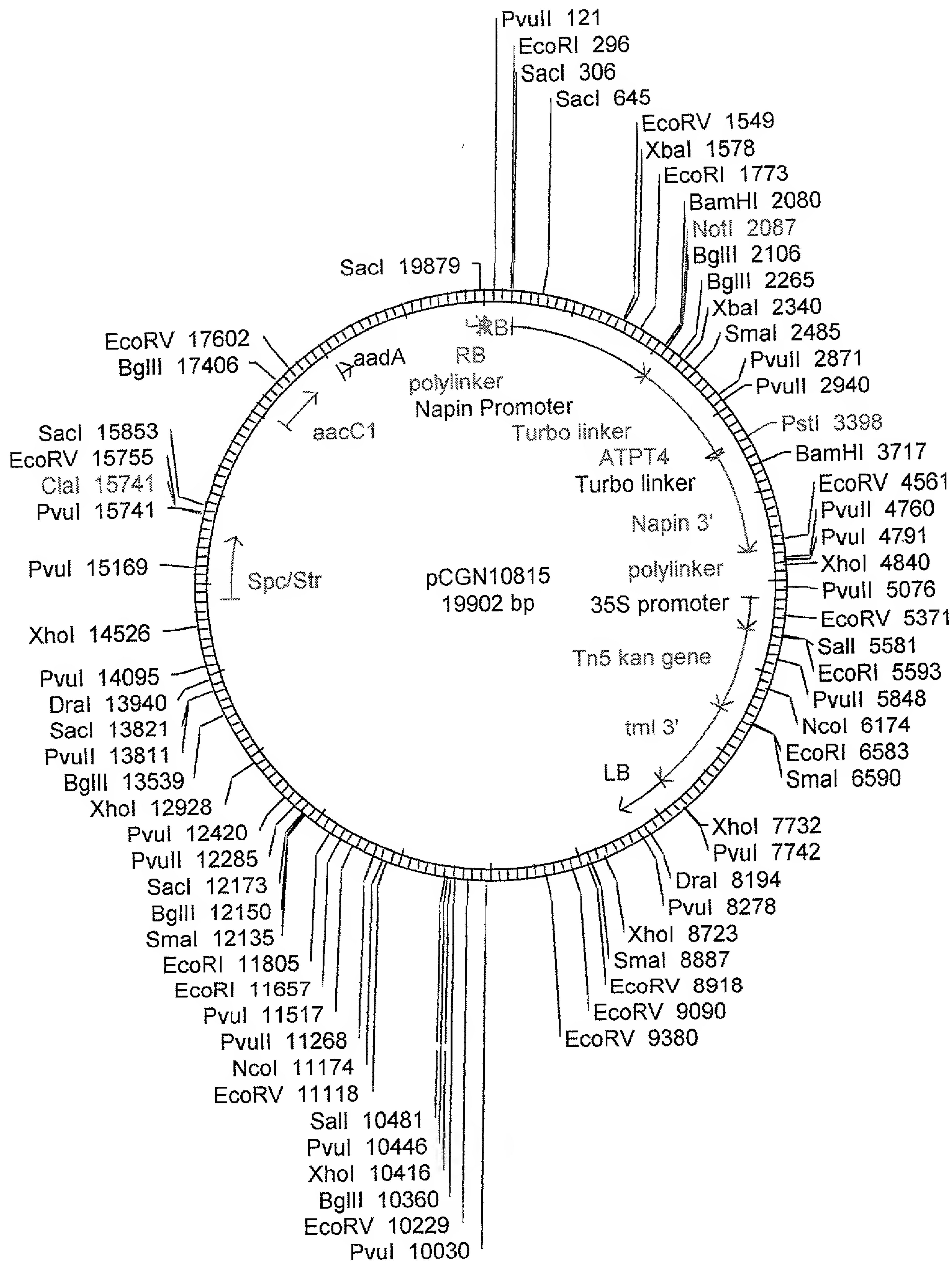
SacI 306
SacI 645
EcoRV 1549
XbaI 1578
EcoRI 1773
SalI 2075
PstI 2085
PvuII 2539
PvuII 2608
SmaI 2994
XbaI 3135
BglII 3210
BglII 3369
NotI 3388
BamHI 3395
XhoI 3400
BamHI 3717
EcoRV 4561
PvuII 4760
PvuI 4791
XhoI 4840
PvuI 5076
EcoRV 5371
SalI 5581
EcoRI 5593
PvuII 5848
NcoI 6174
EcoRI 6583
SmaI 6590
XhoI 7732
PvuI 7742
DraI 8194
PvuI 8278
XhoI 8723
SmaI 8887
EcoRV 8918
EcoRV 9090
EcoRV 9380
PvuI 10030
EcoRV 10229
BglII 10360
XhoI 10416
PvuI 10446
SalI 10481
EcoRV 11118
NcoI 11174
PvuII 11268
PvuI 11517
EcoRI 11657
EcoRI 11805
SmaI 12135
BglII 12150
SacI 12173
PvuII 12285
PvuI 12420
XhoI 12928
BglII 13539
PvuII 13811
SacI 13821
DraI 13940
PvuI 14095
XhoI 14526
PvuI 15169
PvuI 15741
ClaI 15741
EcoRV 15755
SacI 15853
BglII 17406
EcoRV 17602
SacI 19879
RB
polylinker
Napin Promoter
Turbo linker
ATPT4
Turbo linker
Napin 3'
35S promoter
Tn5 kan gene
tml 3'
LB
ori pRi
Spc/Str
aacC1
aadA
pCGN10814
19902 bp

Figure 13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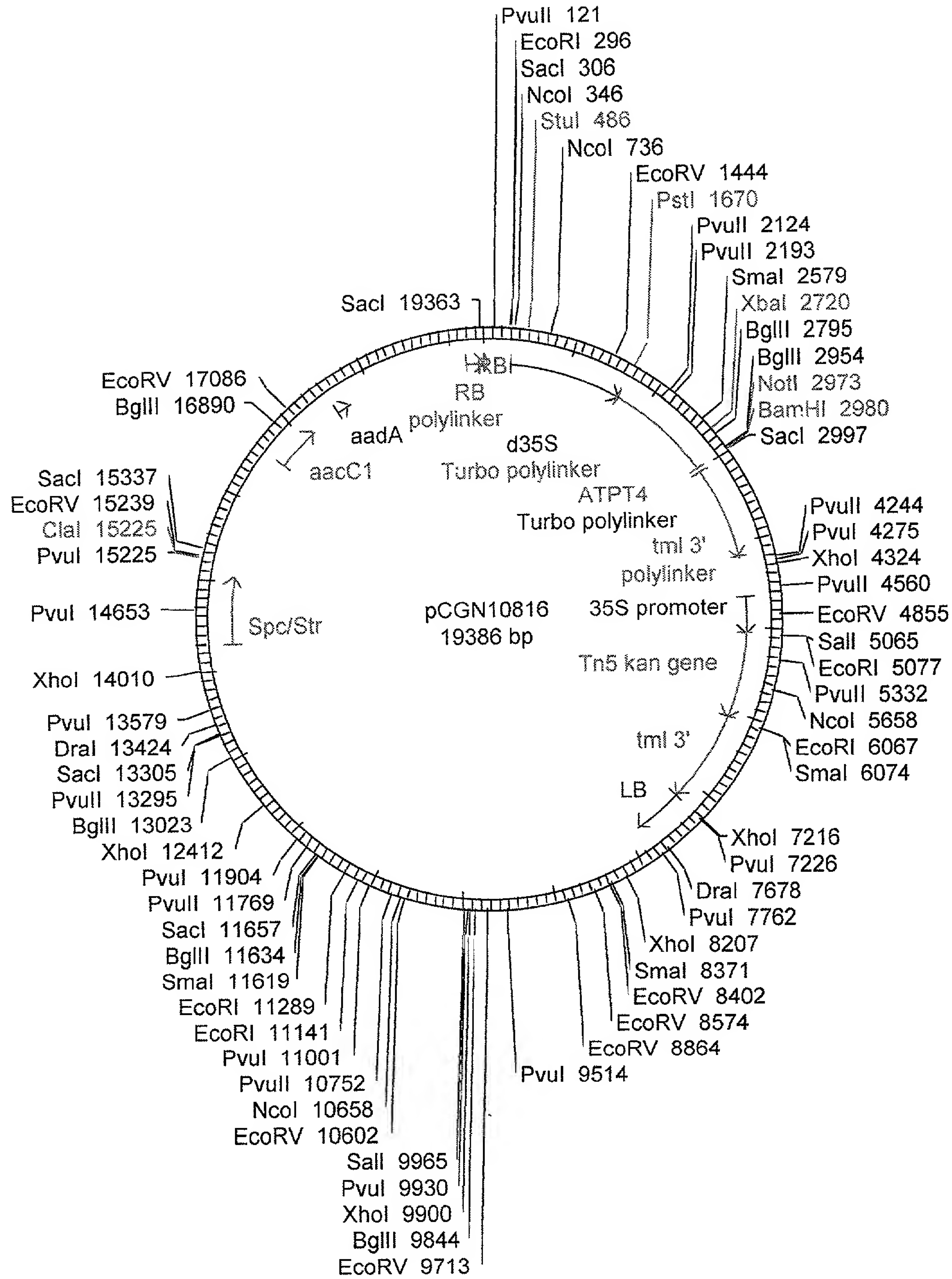
EcoRI 296
SacI 306
SacI 645
EcoRV 1549
XbaI 1578
EcoRI 1773
BamHI 2080
NotI 2087
BglII 2106
BglII 2265
XbaI 2340
SmaI 2485
PvuII 2871
PvuII 2940
PstI 3398
BamHI 3717
EcoRV 4561
PvuII 4760
PvuI 4791
XhoI 4840
PvuII 5076
EcoRV 5371
SalI 5581
EcoRI 5593
PvuII 5848
NcoI 6174
EcoRI 6583
SmaI 6590
XhoI 7732
PvuI 7742
DraI 8194
PvuI 8278
XhoI 8723
SmaI 8887
EcoRV 8918
EcoRV 9090
EcoRV 9380
PvuI 10030
EcoRV 10229
BglII 10360
XhoI 10416
PvuI 10446
SalI 10481
EcoRV 11118
NcoI 11174
PvuII 11268
PvuI 11517
EcoRI 11657
EcoRI 11805
SmaI 12135
BglII 12150
SacI 12173
PvuII 12285
PvuI 12420
XhoI 12928
BglII 13539
PvuII 13811
SacI 13821
DraI 13940
PvuI 14095
XhoI 14526
PvuI 15169
PvuI 15741
ClaI 15741
EcoRV 15755
SacI 15853
BglII 17406
EcoRV 17602
SacI 19879
RB
aadA
polylinker
Napin Promoter
aacC1
Turbo linker
ATPT4
Turbo linker
Napin 3'
polylinker
35S promoter
Spc/Str
pCGN10815
19902 bp
Tn5 kan gene
tml 3'
LB

Figure 14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SacI 306
NcoI 346
StuI 486
NcoI 736
EcoRV 1444
PstI 1670
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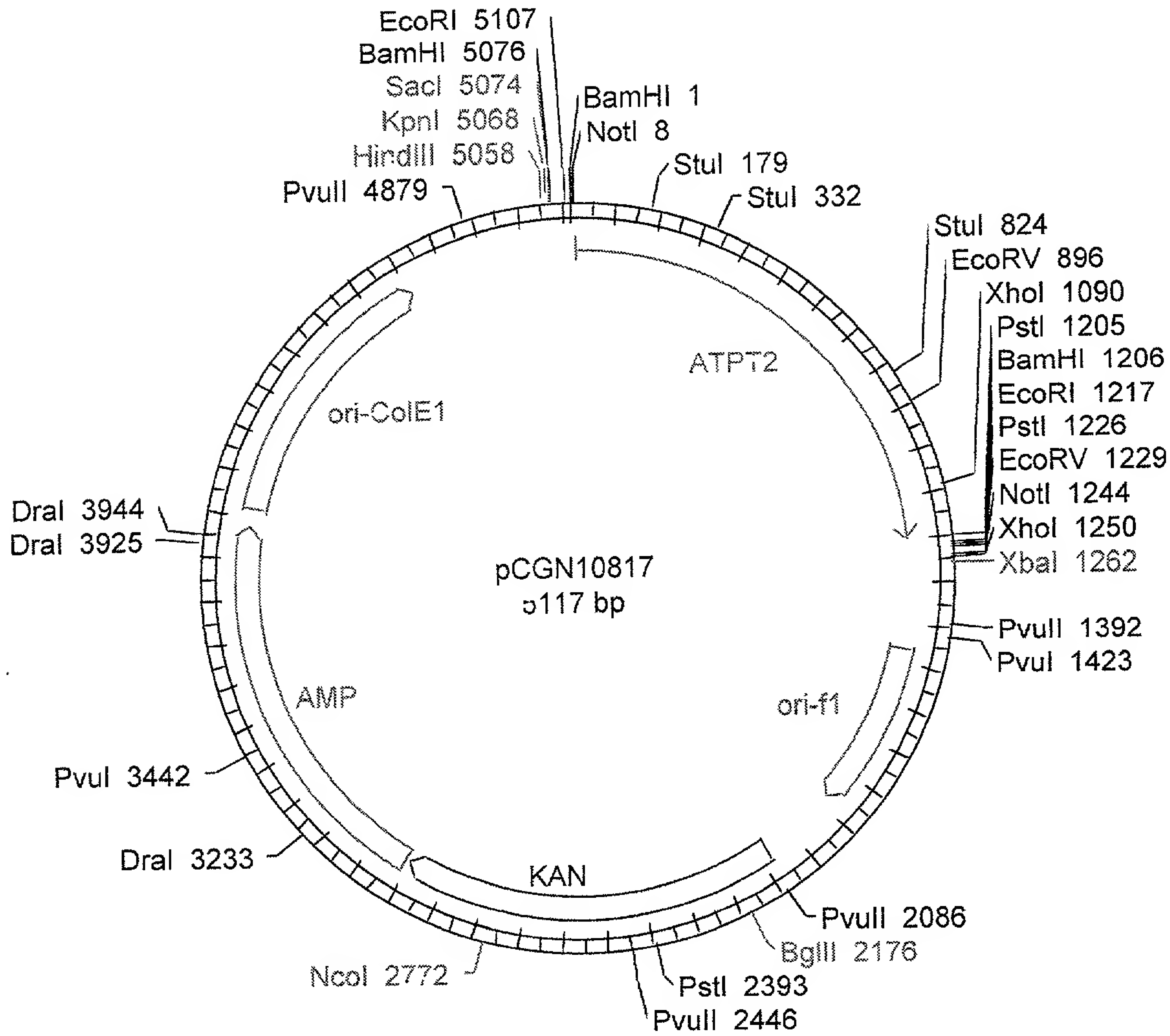
PvuII 2193
SmaI 2579
XbaI 2720
BglII 2795
BglII 2954
NotI 2973
BamHI 2980
SacI 2997
PvuII 4244
PvuI 4275
XhoI 4324
PvuII 4560
EcoRV 4855
SalI 5065
EcoRI 5077
PvuII 5332
NcoI 5658
EcoRI 6067
SmaI 6074
XhoI 7216
PvuI 7226
DraI 7678
PvuI 7762
XhoI 8207
SmaI 8371
EcoRV 8402
EcoRV 8574
EcoRV 8864
PvuI 9514
EcoRV 9713
BglII 9844
XhoI 9900
PvuI 9930
SalI 9965
EcoRV 10602
NcoI 10658
PvuII 10752
PvuI 11001
EcoRI 11141
EcoRI 11289
SmaI 11619
BglII 11634
SacI 11657
PvuII 11769
PvuI 11904
XhoI 12412
BglII 13023
PvuII 13295
SacI 13305
DraI 13424
PvuI 13579
XhoI 14010
PvuI 14653
PvuI 15225
ClaI 15225
EcoRV 15239
SacI 15337
BglII 16890
EcoRV 17086
SacI 19363
RB
RB
polylinker
aadA
d35S
aacC1
Turbo polylinker
ATPT4
Turbo polylinker
tml 3'
polylinker
35S promoter
Spc/Str
pCGN10816
19386 bp
Tn5 kan gene
tml 3'
LB

Figure 15



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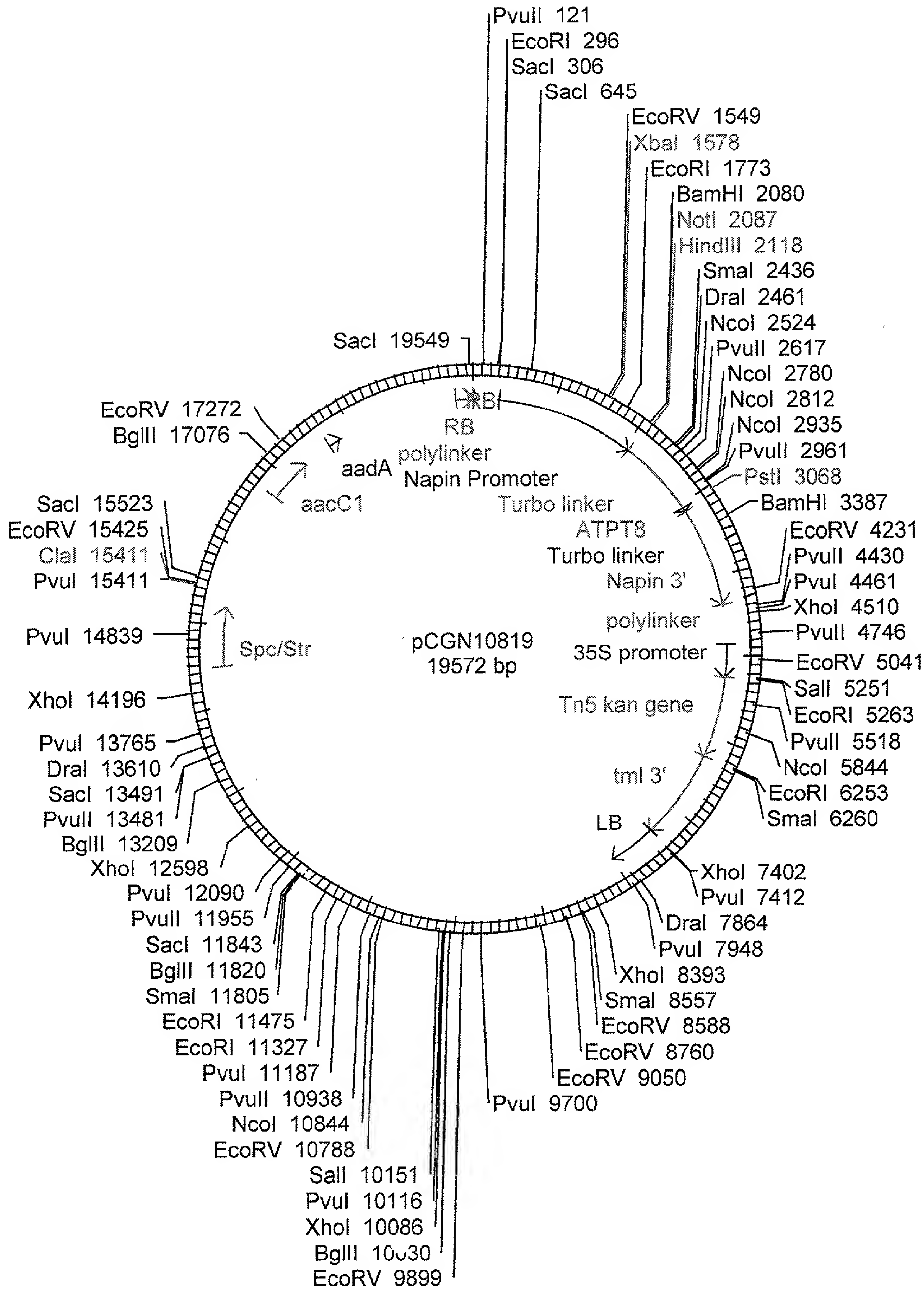
SacI 5074
KpnI 5068
HindIII 5058
PvuII 4879
BamHI 1
NotI 8
StuI 179
StuI 332
StuI 824
EcoRV 896
XhoI 1090
PstI 1205
BamHI 1206
EcoRI 1217
PstI 1226
EcoRV 1229
NotI 1244
XhoI 1250
XbaI 1262
PvuII 1392
PvuI 1423
ATPT2
ori-ColE1
pCGN10817
5117 bp
DraI 3944
DraI 3925
AMP
ori-f1
PvuI 3442
DraI 3233
KAN
PvuII 2086
BglII 2176
NcoI 2772
PstI 2393
PvuII 2446

Figure 16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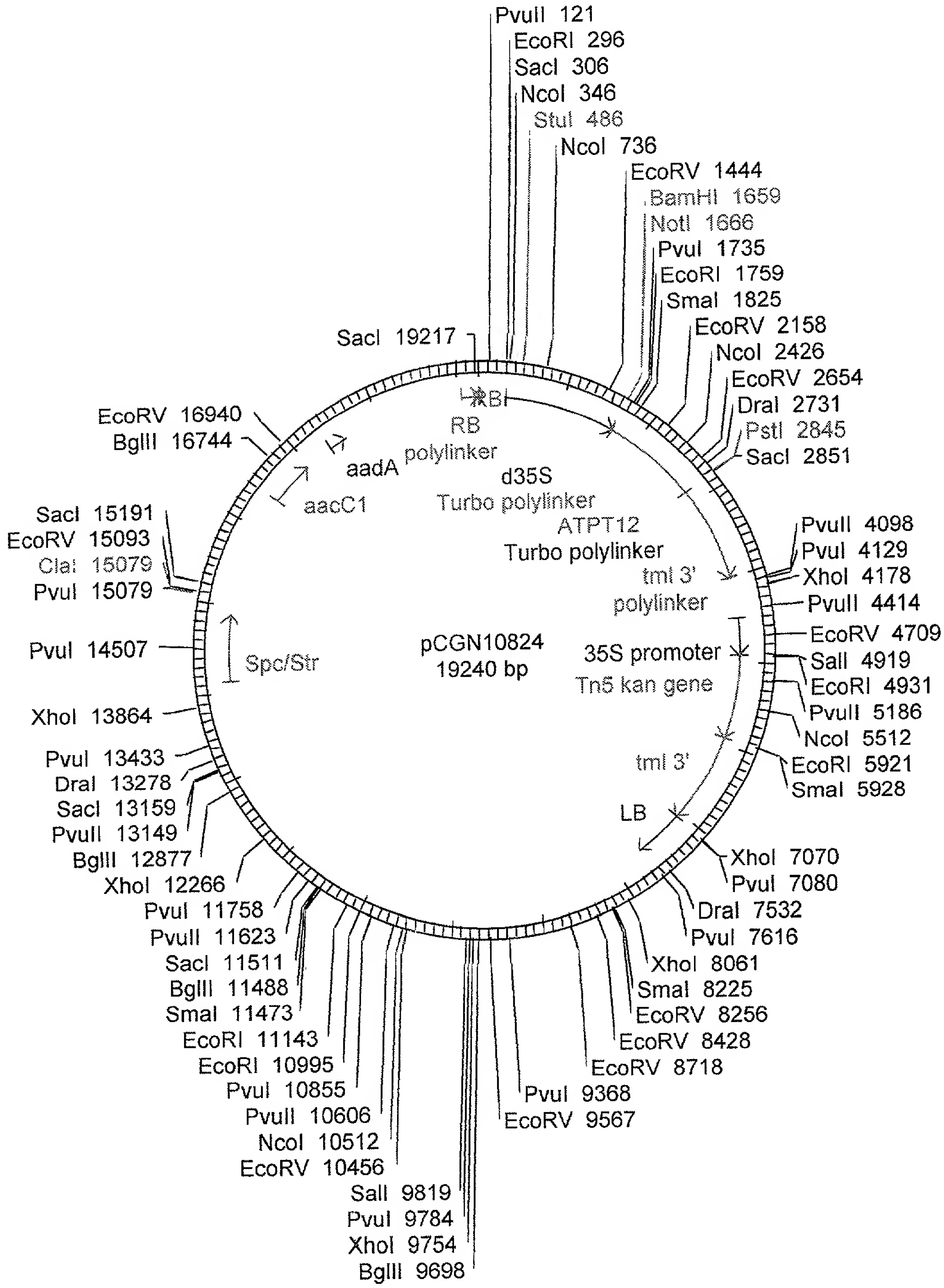
SacI 306
SacI 645
EcoRV 1549
XbaI 1578
EcoRI 1773
BamHI 2080
NotI 2087
HindIII 2118
SmaI 2436
DraI 2461
NcoI 2524
PvuII 2617
NcoI 2780
NcoI 2812
NcoI 2935
PvuII 2961
PstI 3068
BamHI 3387
EcoRV 4231
PvuII 4430
PvuI 4461
XhoI 4510
PvuII 4746
EcoRV 5041
SalI 5251
EcoRI 5263
PvuII 5518
NcoI 5844
EcoRI 6253
SmaI 6260
XhoI 7402
PvuI 7412
DraI 7864
PvuI 7948
XhoI 8393
SmaI 8557
EcoRV 8588
EcoRV 8760
EcoRV 9050
PvuI 9700
EcoRV 9899
BglII 10030
XhoI 10086
PvuI 10116
SalI 10151
EcoRV 10788
NcoI 10844
PvuII 10938
PvuI 11187
EcoRI 11327
EcoRI 11475
SmaI 11805
BglII 11820
SacI 11843
PvuII 11955
PvuI 12090
XhoI 12598
BglII 13209
PvuII 13481
SacI 13491
DraI 13610
PvuI 13765
XhoI 14196
PvuI 14839
PvuI 15411
ClaI 15411
EcoRV 15425
SacI 15523
BglII 17076
EcoRV 17272
SacI 19549
RB
polylinker
Napin Promoter
Turbo linker
ATPT8
Turbo linker
Napin 3'
polylinker
35S promoter
Tn5 kan gene
tml 3'
LB
aadA
aacC1
Spc/Str
pCGN10819
19572 bp

Figure 17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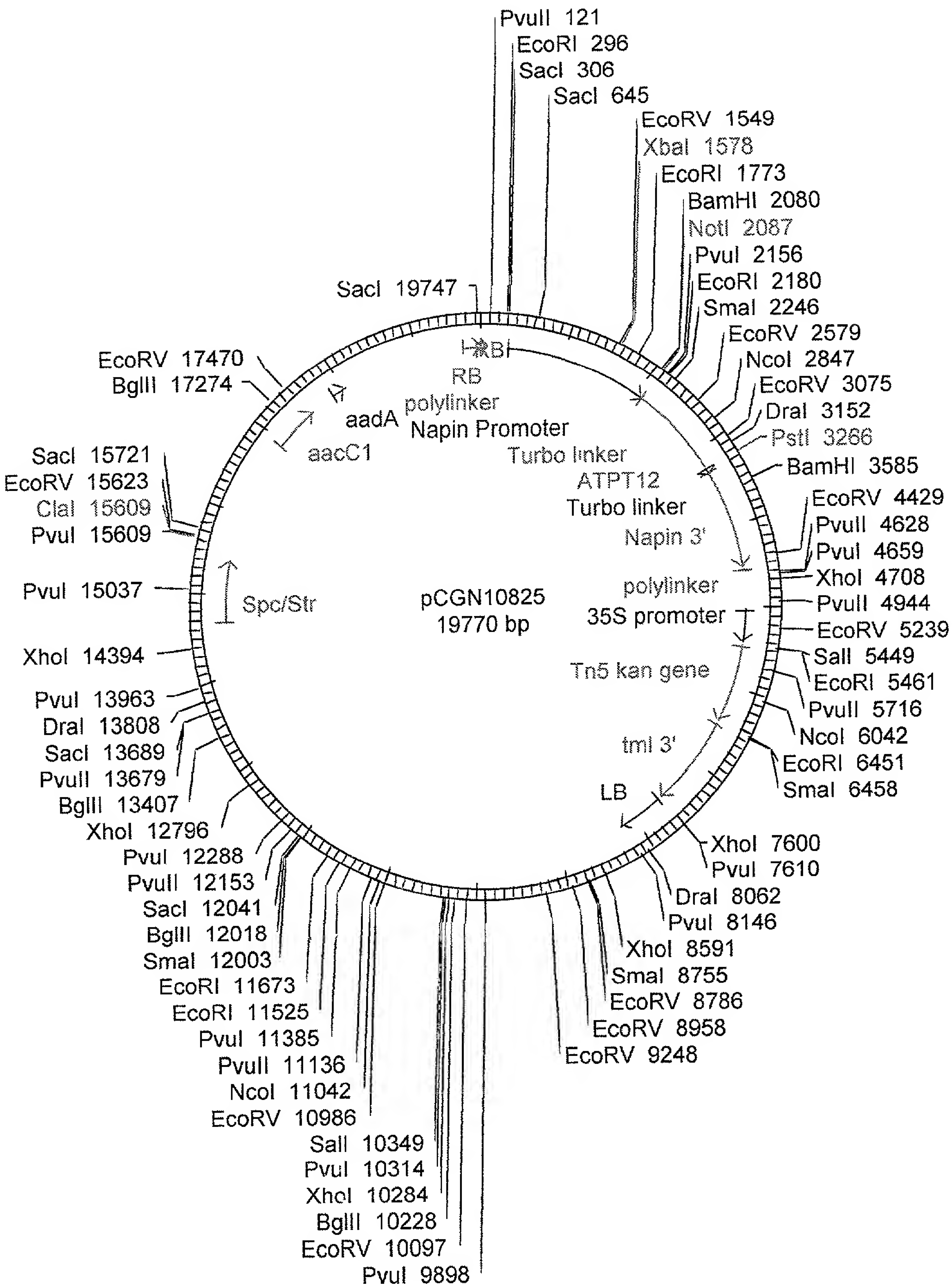
EcoRI 296
SacI 306
NcoI 346
StuI 486
NcoI 736
EcoRV 1444
BamHI 1659
NotI 1666
PvuI 1735
EcoRI 1759
SmaI 1825
EcoRV 2158
NcoI 2426
EcoRV 2654
DraI 2731
PstI 2845
SacI 2851
PvuII 4098
PvuI 4129
XhoI 4178
PvuII 4414
EcoRV 4709
SalI 4919
EcoRI 4931
PvuII 5186
NcoI 5512
EcoRI 5921
SmaI 5928
XhoI 7070
PvuI 7080
DraI 7532
PvuI 7616
XhoI 8061
SmaI 8225
EcoRV 8256
EcoRV 8428
EcoRV 8718
PvuI 9368
EcoRV 9567
SalI 9819
PvuI 9784
XhoI 9754
BglII 9698
EcoRV 10456
NcoI 10512
PvuII 10606
PvuI 10855
EcoRI 10995
EcoRI 11143
SmaI 11473
BglII 11488
SacI 11511
PvuII 11623
PvuI 11758
XhoI 12266
BglII 12877
PvuII 13149
SacI 13159
DraI 13278
PvuI 13433
XhoI 13864
PvuI 14507
PvuI 15079
ClaI 15079
EcoRV 15093
SacI 15191
BglII 16744
EcoRV 16940
SacI 19217
RB
RB polylinker
d35S
Turbo polylinker
ATPT12
Turbo polylinker
tml 3'
polylinker
35S promoter
Tn5 kan gene
tml 3'
LB
aadA
aacC1
Spc/Str
pCGN10824
19240 bp

Figure 18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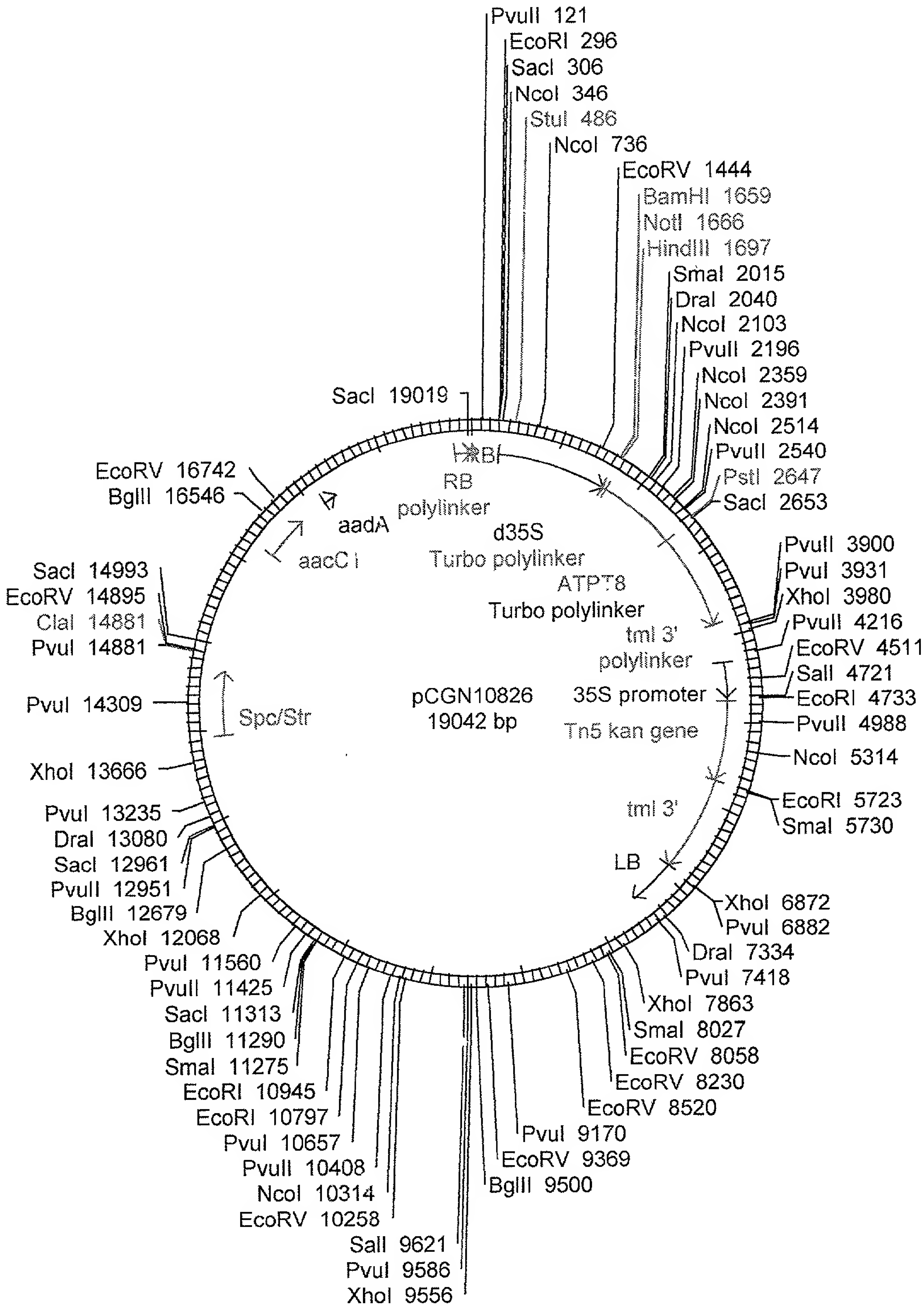
EcoRI 296
SacI 306
SacI 645
EcoRV 1549
XbaI 1578
EcoRI 1773
BamHI 2080
NotI 2087
PvuI 2156
EcoRI 2180
SmaI 2246
EcoRV 2579
NcoI 2847
EcoRV 3075
DraI 3152
PstI 3266
BamHI 3585
EcoRV 4429
PvuII 4628
PvuI 4659
XhoI 4708
PvuII 4944
EcoRV 5239
SalI 5449
EcoRI 5461
PvuII 5716
NcoI 6042
EcoRI 6451
SmaI 6458
XhoI 7600
PvuI 7610
DraI 8062
PvuI 8146
XhoI 8591
SmaI 8755
EcoRV 8786
EcoRV 8958
EcoRV 9248
PvuI 9898
EcoRV 10097
BglII 10228
XhoI 10284
PvuI 10314
SalI 10349
EcoRV 10986
NcoI 11042
PvuII 11136
PvuI 11385
EcoRI 11525
EcoRI 11673
SmaI 12003
BglII 12018
SacI 12041
PvuII 12153
PvuI 12288
XhoI 12796
BglII 13407
PvuII 13679
SacI 13689
DraI 13808
PvuI 13963
XhoI 14394
PvuI 15037
PvuI 15609
ClaI 15609
EcoRV 15623
SacI 15721
BglII 17274
EcoRV 17470
SacI 19747
RB
polylinker
Napin Promoter
Turbo linker
ATPT12
Turbo linker
Napin 3'
polylinker
35S promoter
Tn5 kan gene
tml 3'
LB
aadA
aacC1
Spc/Str
pCGN10825
19770 bp

Figure 19



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SacI 306
NcoI 346
StuI 486
NcoI 736
EcoRV 1444
BamHI 1659
NotI 1666
HindIII 1697
SmaI 2015
DraI 2040
NcoI 2103
PvuII 2196
NcoI 2359
NcoI 2391
NcoI 2514
PvuII 2540
PstI 2647
SacI 2653
PvuII 3900
PvuI 3931
XhoI 3980
PvuII 4216
EcoRV 4511
SalI 4721
EcoRI 4733
PvuII 4988
NcoI 5314
EcoRI 5723
SmaI 5730
XhoI 6872
PvuI 6882
DraI 7334
PvuI 7418
XhoI 7863
SmaI 8027
EcoRV 8058
EcoRV 8230
EcoRV 8520
PvuI 9170
EcoRV 9369
BglII 9500
SalI 9621
PvuI 9586
XhoI 9556
EcoRV 10258
NcoI 10314
PvuII 10408
PvuI 10657
EcoRI 10797
EcoRI 10945
SmaI 11275
BglII 11290
SacI 11313
PvuII 11425
PvuI 11560
XhoI 12068
BglII 12679
PvuII 12951
SacI 12961
DraI 13080
PvuI 13235
XhoI 13666
PvuI 14309
PvuI 14881
ClaI 14881
EcoRV 14895
SacI 14993
BglII 16546
EcoRV 16742
SacI 19019
RB
polylinker
aadA
aacC i
d35S
Turbo polylinker
ATPT8
Turbo polylinker
tml 3'
polylinker
35S promoter
Tn5 kan gene
tml 3'
LB
Spc/Str
pCGN10826
19042 bp

Figure 20

```
                    *        20         *        40         *        60         *        80         *
SLR1736 : MATIQAFWR-----FSRPHTI GTTLS------- WA YL TILGD--G-NSVNSPAS----------- DL FGAWLACLLGNVYIVG  :  64
SLR0926 : MVAQTPSS---PPLWLTIIYL RWHK ------AGRL LM PALWA-- CLAAQ LPP----------- PL GTIALGTLATSGLGCV  :  68
SLL1899 : MVTSTKIHRQHDSMGAVCKSYYQLTK ------R IP LL TTAAS-- WIASE RVD----------- PK LITLLGGTLAAASAQT  :  71
SLR0056 : MSDTQNTGQ--NQAKARQLLG KGAA GESSIWK RLQLMKPITWIPL WGVVC AASSGGYIWSVEDF KA TCMLLSGPLMTGYTQT  :  88
SLR1518 : MTESSPLAP--STAPATRKLW AAIK ------P YT AV PITVGSA AYGLT QWH-----------GDVFTIFLLSATAIIAWINLS  :  71
          M                     p         6               g               l     L

          100         *       120         *       140         *       160         *       180
SLR1736 : NQ WDVD RINKPNLPLANGDFS AQGRWI GLCG SLAIAWGLGL LGLTVGIS-- IIGTAYSVPPVRLKRFSLLAALCI TVRGI : 152
SLR0926 : ND WDRD PQVERTKQRPLAARA -SVQVG GVA -LLCAAGLAF LTPLS-----FWLCVAAVPVLVAYPGAKRVFVPVQ VL-SI : 150
SLL1899 : NC YDQD YEMLRTRARPIPAGK -QPRHA IFA ALGVLSFALLAT VNVLSGC--- ALSGIVFYMLVYTHWLKRHTAQNI IG-GA : 156
SLR0056 : NDFYDRD AINEPYRPIPSGAIS PQVVTQ LIL GIGVAYGLDV AQHDFPIMMV TLGGAFVAYIYSAPPLKLKQNGWLGNY-AL : 177
SLR1518 : ND FDSDTGIDVRKAHSVVNLTGNRNLVFLISNFF GVLGLMSMSWRAQDWT----V ELIGVAIFLGYTYQGPPFRLGYLG GELIC : 157
          N  5D Did                              l a 6     6               l 6

                 *       200         *       220         *       240         *       260         *
SLR1736 : VVNLGLFLF RIGLGYPPT ITPIW TLFIL F VAIAIFKDVPDM G RQFK Q LTLQIGKQNVFRGTLILLTG-CY AMA WGLWA : 241
SLR0926 : AWGFAV IS S---AVTGD TDATW WGATVFW LGFDTVYAMADR D RRIG N SALFFG-QYVGEA GIFFA--LT GCLFYLGMI : 234
SLL1899 : AGSIPP VG A---AVTGD SWTPW FALIF W PPHFWALALMIKDDYAQVN PMLPVIAGEEKTVSQ WYYSL--LV PFS LLVYP : 241
SLR0056 : GASYIA PW AG-HALFGT NPTIM TLIYS AGLGIAVVNDFKSV G RQLG K LPVMFG-IGTAAW CVIMI--DVFQAG AGYLI : 263
SLR1518 : LITFGP AIAAAYYSQSQSFSWNLLTPSVFVG S AILLFCSHFHQV D LAAGKK PIVRLG-TKLGSQ LTLSVVSLY ITA GVLCH : 246
                  l              l     vl       t              e d         6  G

              280         *       300         *       320         *       340
SLR1736 : AMPLNTAF I SHLCLLA WWRSRDVHLESKTEIASFYQFIWKLFFLEYLLYP A WLPNFSNTIF--------- : 308
SLR0926 : LMLNPLYW S A AIVGW QYIQLSAPT EPKLYGQ--IFGQNVIIGF LLAG L GWL---------------- : 292
SLL1899 : LHQLGILY A A ILGGQF VKAWQLKQA GDRDLARG-LFKFSIFYLM LCLA V DSLPVTHQLVAQMGTLLLG : 316
SLR0056 : YVHQQLYATI L LLIPQ TFQDMYFLRN LENDVKYQ-ASAQPFLVFG LATG A GHAGI-------------- : 324
SLR1518 : QAPWQTLL IAS PWAVQ RHVGQYHDQ EQVSNCKFIAVNLHFFSGMMAAGYGWAGLG--------------- : 307
                                    p                   6
```

Figure 21

```
                     *        20         *        40         *        60         *        80
ATPT2   : ---------MESLLSSSSLVSAAGGFCWKKQNLKLHSLSEIRVLRCDSSKVVAKPKFRNNLVRPDGQGSSLLLYPKHKSRFRVNATAGQ :  80
SLR1736 : ------------------------------------------------------------------------------------ :  -
ATPT3   : MAFFGLSRVSRRLLKSSVSVTPSSSSALLQSQHKSLSNPVTTHYTNPFTKCYPSWNDNYQVWSKGRELHQEKFFGVGWNYRLICGMSSS :  89
SLR0926 : ------------------------------------------------------------------------------------ :  -
ATPT4   : ------MWRRSVVYRFSSRISVSSSLPNPRLIPWSRELCAVNSFSQP----------------PVSTESTAKLGITGVRSDANRVFATA :  67
SLL1899 : -------------------------------------------------------------------------------MVTS :   4
ATPT12  : ---------MTSILNTVSTIHSSRVTSVDRVGVLSLRNSDSVEFT-----------------RRRSGFSTLIYESPGRRFVVRAAETDT :  63
SLR0056 : -------------------------------------------------------------------------------MSDT :   4
ATPT8   : -------------------------------------------------------------------------------MVLA :   4
SLR1518 : -------------------------------------------------------------------------------MTES :   4

            *       100         *       120         *       140         *       160         *        1
ATPT2   : PEAFDSNSKQK----------SFRDS DAFYR------------FSRPHT IGTVLSILS-----VSFLAVEKVS--DISPLLFTGILE : 140
SLR1736 : ------------------------MAT QAFWR------------FSRPHT IGTTLSVWAVY----LLTILGDGN-SVNSPASLDLVFG :  49
ATPT3   : SSVLEGKPKKDDKEKSDGVVVKKASW DLYLPEEVRGYAKLARLDKPIGTWLLAWPCMWS------IALAADPGS--LPSFKYMALFGC : 170
SLR0926 : --MVAQTPSSP------------PLW TIIYL---------LRWHKPAGR ILMIPALWA------VCLAAQ--G--LPPLPELGTIAL :  56
ATPT4   : TAAATATATTG----EISSRVAALAG GHHYAR--------CYWELSKAK SMLVVATSG------TGYILGTGNAAISFPGLCYTCAG : 138
SLL1899 : TKIHRQHDSMG---------AVCKSYYQLTKP------------RIIPLL ITTAASMWI----------ASEGR--VDLPKLLITLLG :  60
ATPT12  : DKVKSQTPDKAP---AGGSSINQLLG KGAS----------QETNKWKIR QLTKPVTWPPLVWGVVCGAAASGNFHWTPEDVAKSILC : 139
SLR0056 : QNT-GQNQAKA----------RQLLG KGAAP---------GESSIWKIR QLMKPITWIPLIWGVVCGAASSGGYIWSVEDFLKALTC :  73
ATPT8   : EVPKLASAAEY----------FFKRG QGKQF------------RSTILL MATALNVRVP------EALIGEST--DIVTSELRVRQR :  63
SLR1518 : SPLAPSTAPAT-------------RKLW AAIKP---------------PMYTVAVVPITVG------SAVAYGLTG--QWHGDVFTIFLL :  59

            80         *       200         *       220         *       240         *       260
ATPT2   : AV AALMMNIYIVG  Q S VE  KVNKPYLPLASGEYSVNTGIAIVASFS MSFWLGWIVGSWPLFWALF SFMLGTAYS-INLPLLR : ?28
SLR1736 : AW ACLLGNVYIVG  Q W VD  RINKPNLPLANGDFSIAQGRWIVGLCG ASLAIAWGLG-LWLGLTVG SLIIGTAY---SVPPVR : 134
ATPT3   : GA L---LRGAGCT  D L QD  TKVDRTKLRPIASGLLT-PFQGIGFLG QLLLGLG-----ILLQLNNYSRVLGAS----SLLLVF : 246
SLR0926 : GT A---TSGLGCV  D W RD  PQVERTKQRPLAARALS-VQVGIGVAL ALLCAAG-----LAFYLTP SFWLCVA----AVPVIV : 132
ATPT4   : TM I----AASANS  Q EEISN SKMKRTMLRPLPSGRISVPHAVAWATIAGASGACL-----LASKTNM AAGLASAN---LVLYAF : 215
SLL1899 : GT A----AASAQT  C Y QD  YEMLRTRARPIPAGKVQPRHALIFALA GVLSFAL-----LATFVNV SGCLALSG---IVFYML : 137
ATPT12  : MM SGPCLTGYTQT  DWY RD  AINEPYRPIPSGAISEPEVITQVWVLL GGLGIAGILD-VWAGHTTPTVFYLALG----GSLLSY : 223
SLR0056 : ML SGPLMTGYTQT  DFY RD  AINEPYRPIPSGAISVPQVVTQILILL AGIGVAYGLD-VWAQHDFP MMVLTLG----GAFVAY : 157
ATPT8   : GIAE---ITEMIHVASL H DV  DADTRRGVGSLNVVMGNKMSVLAGDFL SRACGAL-----AALKNTE VALLATAVEHLVTGETM : 144
SLR1518 : SA A---IIAWINLS D F SDTGIDVRKAHSVVNLTGNRNLVFLISNFFL AGVLGLMSMS--WRAQDWT LELIGVA-----IFLGY : 138
                         n   d   d                                                  6
```

Figure 22 1/2

09689069 .101400

```
                *       280         *       300         *       320         *       340         *
ATPT2    : WKR-FALVAAMCILAVRAIIVQIAFYLH-IQTHVFGRPIFFTRPLIFATAFMSFFSVVIALFKDIPDIEGDKI-FGIRSFSVTLG-QKR : 313
SLR1736  : LKR-FSLLAALCILTVRGIVVNLGLFLF-FRIGLGYPPTLITPIWV-LTLFILVFTVAIAIFKDVPDMEGDRQ-FKIQTLTLQIG-KQN : 218
ATPT3    : SYP-LMKRFTFWPQAFLGLTINWGALLG--WT--AVKGSIAPSIVLPLYLSGVCWTIVYDTIYAHQDKEDDVK-VGVKSTALRFG-DNT : 328
SLR0926  : AYP-GAKRVFPVPQLVLSIAWGFAVLIS--WS--AVTGDLTDATWV-LWGATVFWTLGFDTVYAMADREDDRR-IGVNSSALFFG-QYV : 213
ATPT4    : VYT-PLKQLHPINTWVGAVVGAIPPLLG--WA--AASGQISYNSMV-LPAALYFWQIPHFMALAHLCRNDYAA-GGYKMLSLFDP---S : 294
SLL1899  : VYTHWLKRHTAQNIVIGGAAGSIPPLVG--WA--AVTGDLSWTPWV-LFALIFLWTPPHFWALALMIKDDYAQ-VNVPMLPVIAGEEKT : 220
ATPT12   : IYS-APPLKLKQNGWVGNFALGASYISLPWWAGQALFGTLTPDVVV-LTLLYSIAGLGIATVNDFKSVEGDRA-LGLQSLPVAFG-TET : 308
SLR0056  : IYS-APPLKLKQNGWLGNYALGASYIALPWWAGHALFGTLNPTIMV-LTLIYSLAGLGIAVVNDFKSVEGDRQ-LGLKSLPVMFG-IGT : 242
ATPT8    : EITSSTEQRYSMDYYVQKTYYKTASLISNSCKAVAVLTGQTAEVAVLAFEYGRNLGLAFQLIDDILDFTGTSASLGKGSLSDIRH--GV : 231
SLR1518  : TYQGPPFRLGYLGLGELICLITFGPLAI-AAAYYSQSQSFSWNLLT-PSVFVGISTAIILFCSHFHQVEDDLA-AGKKSPIVRLG-TKL : 223
                                                                                   g       g

         360         *       380         *       400         *       420         *       440
ATPT2    : VFWTCVTLLQMAYAVAILVGATSPFIWSKVISVVGHVILATTLWARAKSVDLSSKTEITS---CYMFIWKLFYAEYLLLPFLK------ : 393
SLR1736  : VFRGTLILLTGCYLAMAIWGLWAAMPLNTAFLIVSHLCLLALLWWRSRDVHLESKTEIAS---FYQFIWKLFFLEYLLYPLALWLPNFS : 304
ATPT3    : KLWLTGFGTASIGFLALSGFSADLGWQYYASLAAASGQLGWQIGTADLSSGADCS---------RKFVSNKWFGAIIFSGVVLGRSFQ- : 407
SLR0926  : GEAVGIFFALTIGCLFYLGMILMLNPLYWLSLAIAI--VGWVIQYIQLSAPTPEP-KLY----GQIFGQNVIIGFVLLAGLLLGWL--- : 292
ATPT4    : GKRIAAVALRNCFYMIPLGFIAYDWGLTSSWFCLESTLLTLAIAATAFSFYRDRTMHKA----RKMFHASLLFLPVFMSGLLLHRVSND : 379
SLL1899  : VSQIWYYSLLVVPFSLLLVYPLHQLGILYLAIAIIL--GGQFLVKAWQLKQAPGDRDLA----RGLFKFSIFYLMLLCLAVVIDSLPVT : 303
ATPT12   : AKWICVGAIDITQLSVAGYLLASGKPYYALALVALI--IPQIVFQFKYFLKDPVKYDVK----YQASAQPFLVLGIFVTALASQH---- : 387
SLR0056  : AAWICVIMVDVFQAGIAGYLIYVHQQLYATIVLLLL--IPQITFQDMYFLRNPLENDVK----YQASAQPFLVFGMLATGLALGHAGI- : 324
ATPT8    : ITAPILFAMEEFPQLREVVDQVEKDPRNVDIALEYLGKSKGIQRARELAMEHANLAAAAIGSLPETDNEDVKRSRRALIDLTHRVITRN : 320
SLR1518  : GSQVLTLSLVSLYLITAIGVLCHQAPWQTLL.IASLPWAVQLLRHVGQYHDQPEQVSNCK---FIAVNLHFFSGMLMA/.GYGWAGLG-- : 307

           *       460         *       480         *
ATPT2    : ------------------------------------------------- :  -
SLR1736  : NTIF--------------------------------------------- : 308
ATPT3    : ------------------------------------------------- :  -
SLR0926  : ------------------------------------------------- :  -
ATPT4    : NQQQLVEEAGLTNSVSGEVKTQRRKKRVAQPPVAYASAAPFPFLPAPSFYSP : 431
SLL1899  : --HQLVAQMGTLLLG---------------------------------- : 316
ATPT12   : ------------------------------------------------- :  -
SLR0056  : ------------------------------------------------- :  -
ATPT8    : K------------------------------------------------ : 321
SLR1518  : ------------------------------------------------- :  -
```

Figure 22 2/2

09658069 .101400

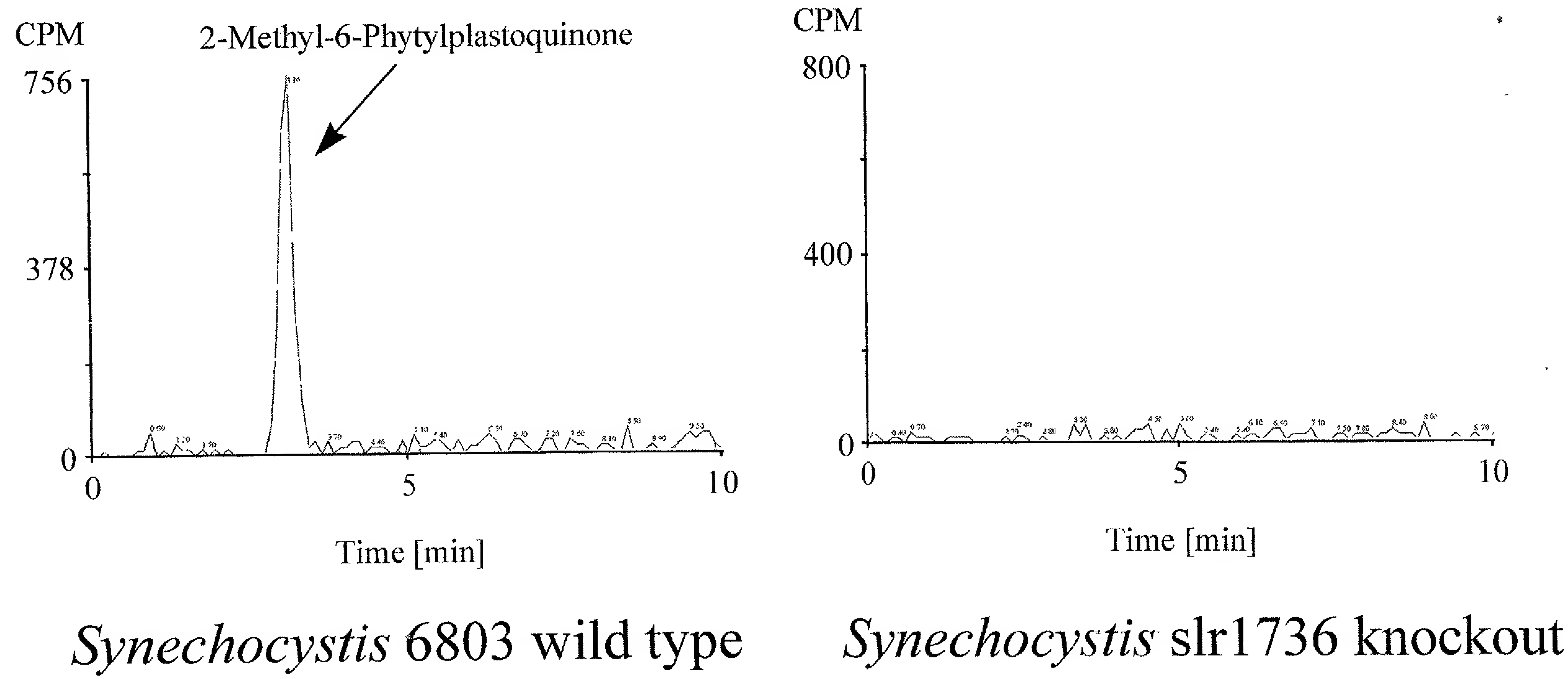
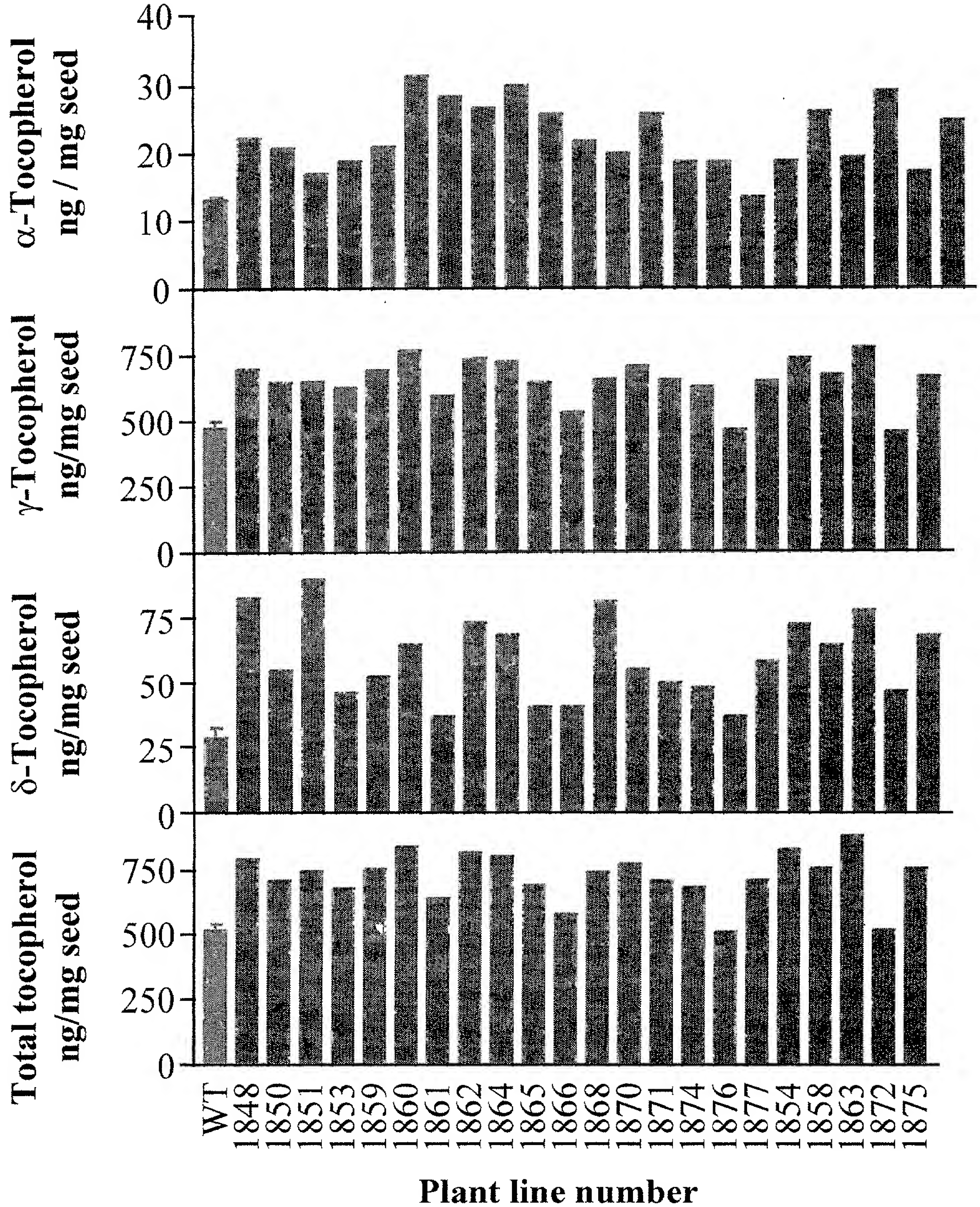

CPM
2-Methyl-6-Phytylplastoquinone
756
378
0
0
5
10
Time [min]
Synechocystis 6803 wild type
CPM
800
400
0
0
5
10
Time [min]
Synechocystis slr1736 knockout

Figure 23



α-Tocopherol ng / mg seed
γ-Tocopherol ng/mg seed
δ-Tocopherol ng/mg seed
Total tocopherol ng/mg seed
0
10
20
30
40
250
500
750
25
50
75
WT
1848
1850
1851
1853
1859
1860
1861
1862
1864
1865
1866
1868
1870
1871
1874
1876
1877
1854
1858
1863
1872
1875
Plant line number

Figure 24

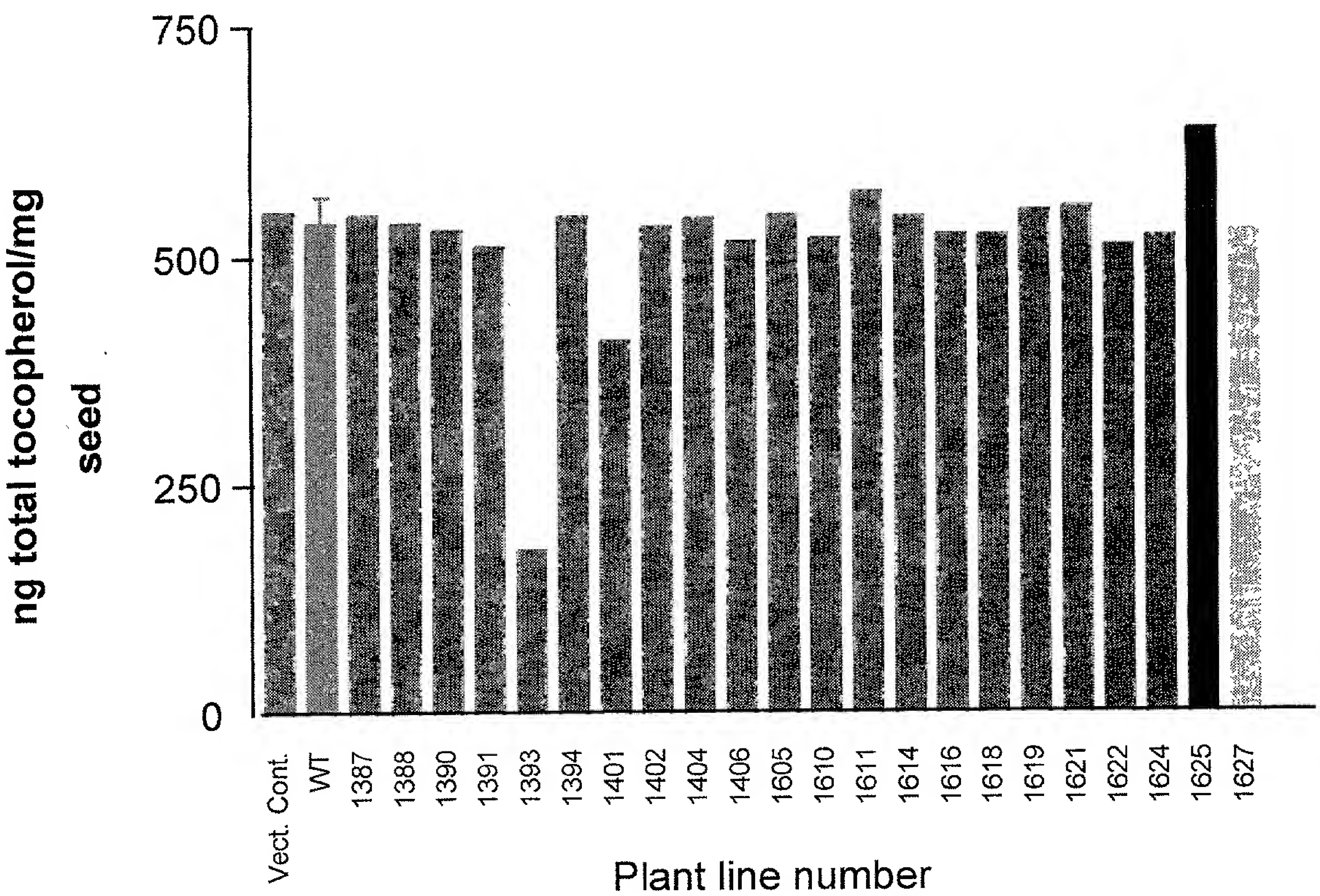

750
500
250
0
ng total tocopherol/mg seed
Vect. Cont.
WT
1387
1388
1390
1391
1393
1394
1401
1402
1404
1406
1605
1610
1611
1614
1616
1618
1619
1621
1622
1624
1625
1627
Plant line number

Figure 25

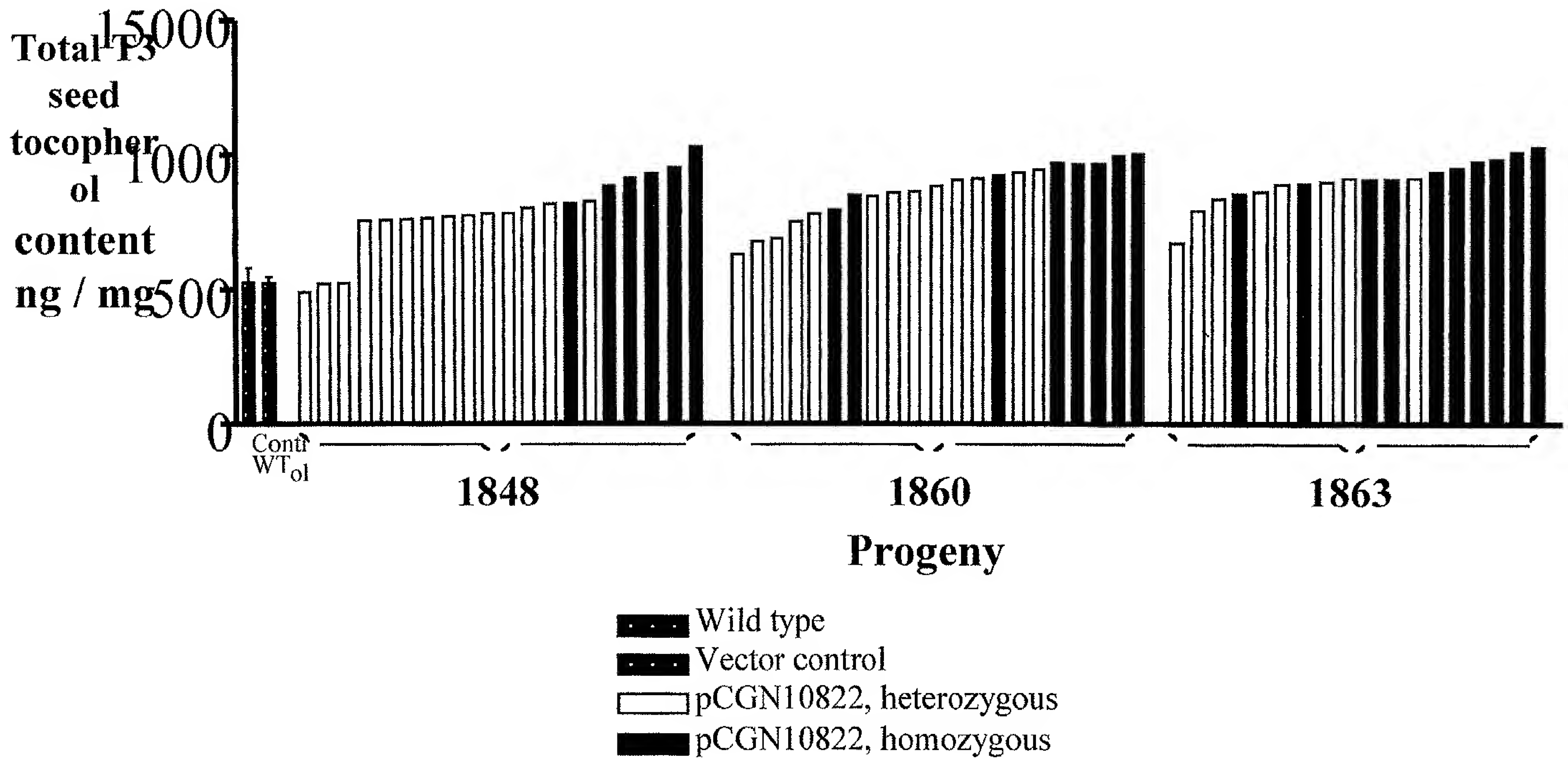

Total T3 seed tocopherol content ng / mg
1500
1000
500
0
Contr
WT
ol
1848
1860
1863
Progeny
Wild type
Vector control
pCGN10822, heterozygous
pCGN10822, homozygous

Figure 26

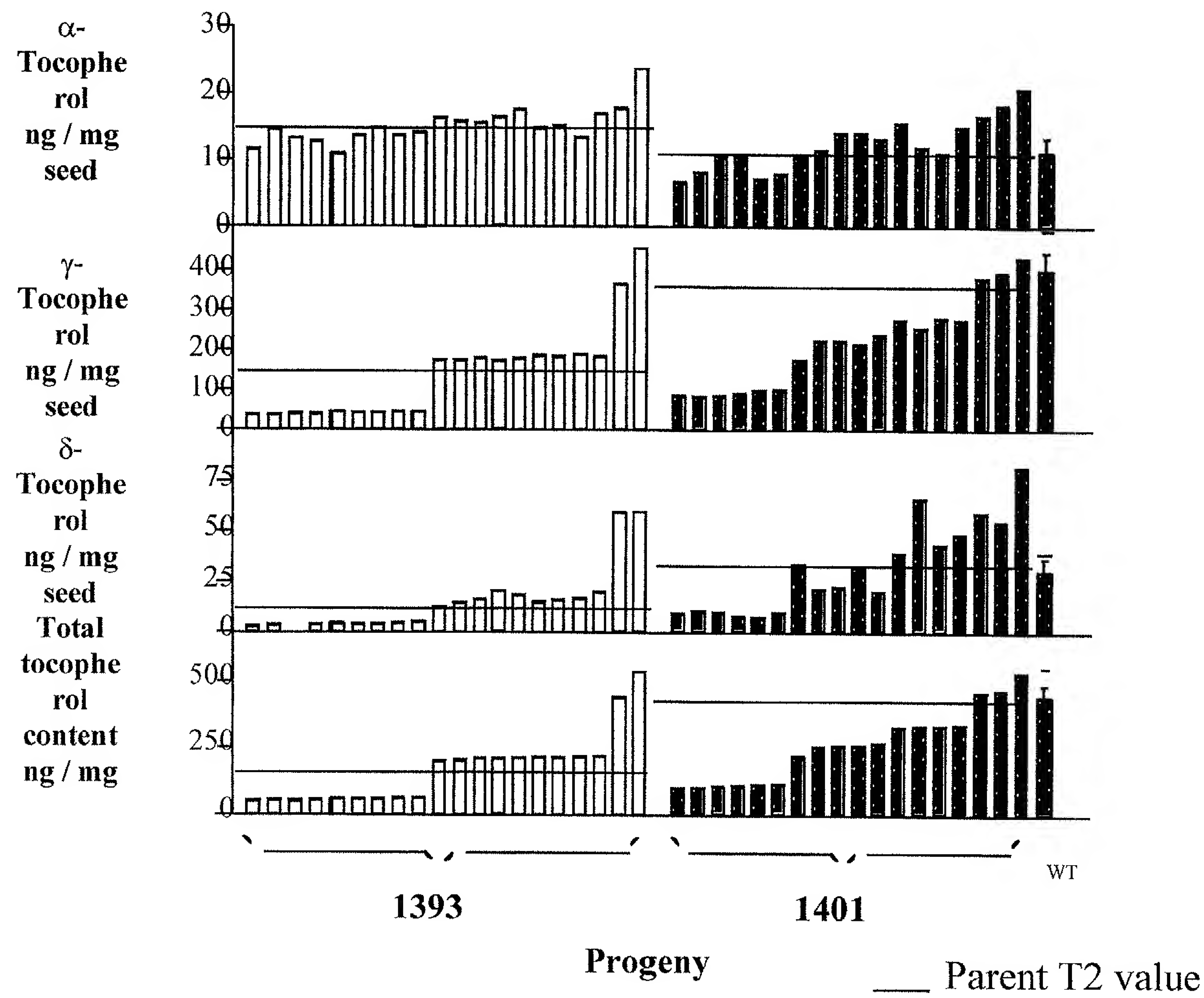


Figure 27

09689069 .101400

Total tocopherol in Napin ATPT2 Canola Seed

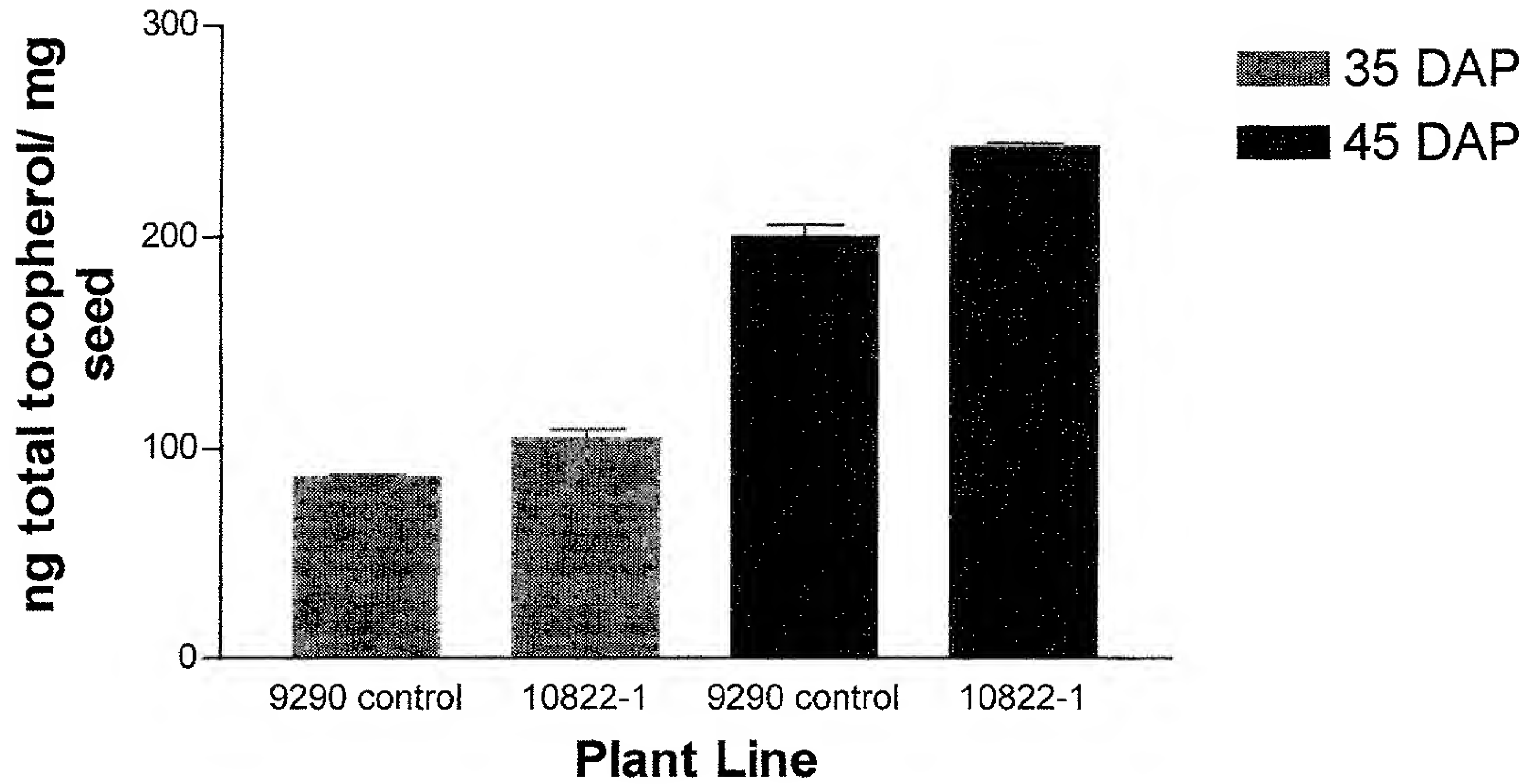
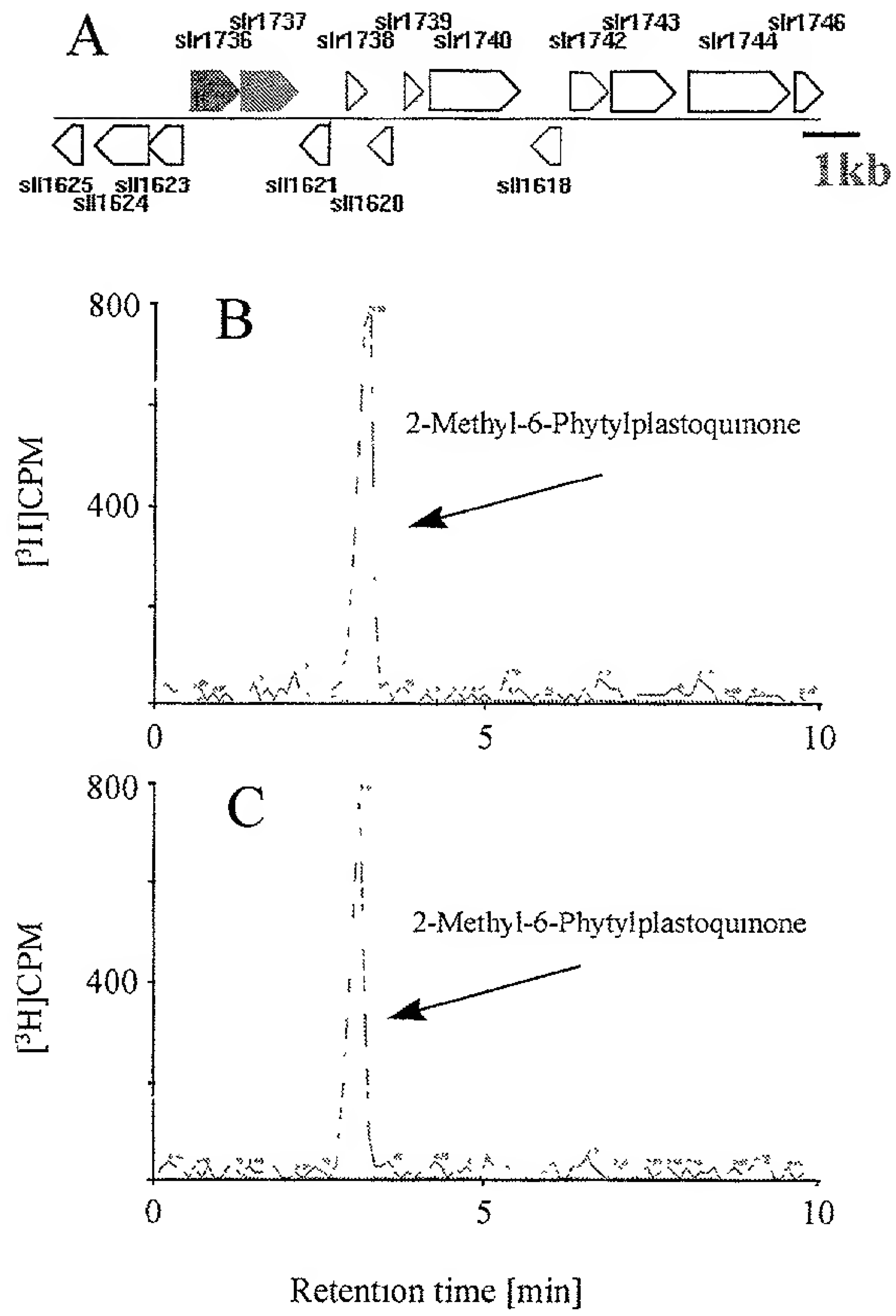


Figure 28



A
slr1736
slr1737
slr1738
slr1739
slr1740
slr1742
slr1743
slr1744
slr1746
sll1625
sll1624
sll1623
sll1621
sll1620
sll1618
1kb
B
800
400
[³H]CPM
2-Methyl-6-Phytylplastoquinone
0
5
10
C
800
400
[³H]CPM
2-Methyl-6-Phytylplastoquinone
0
5
10
Retention time [min]

Figure 29

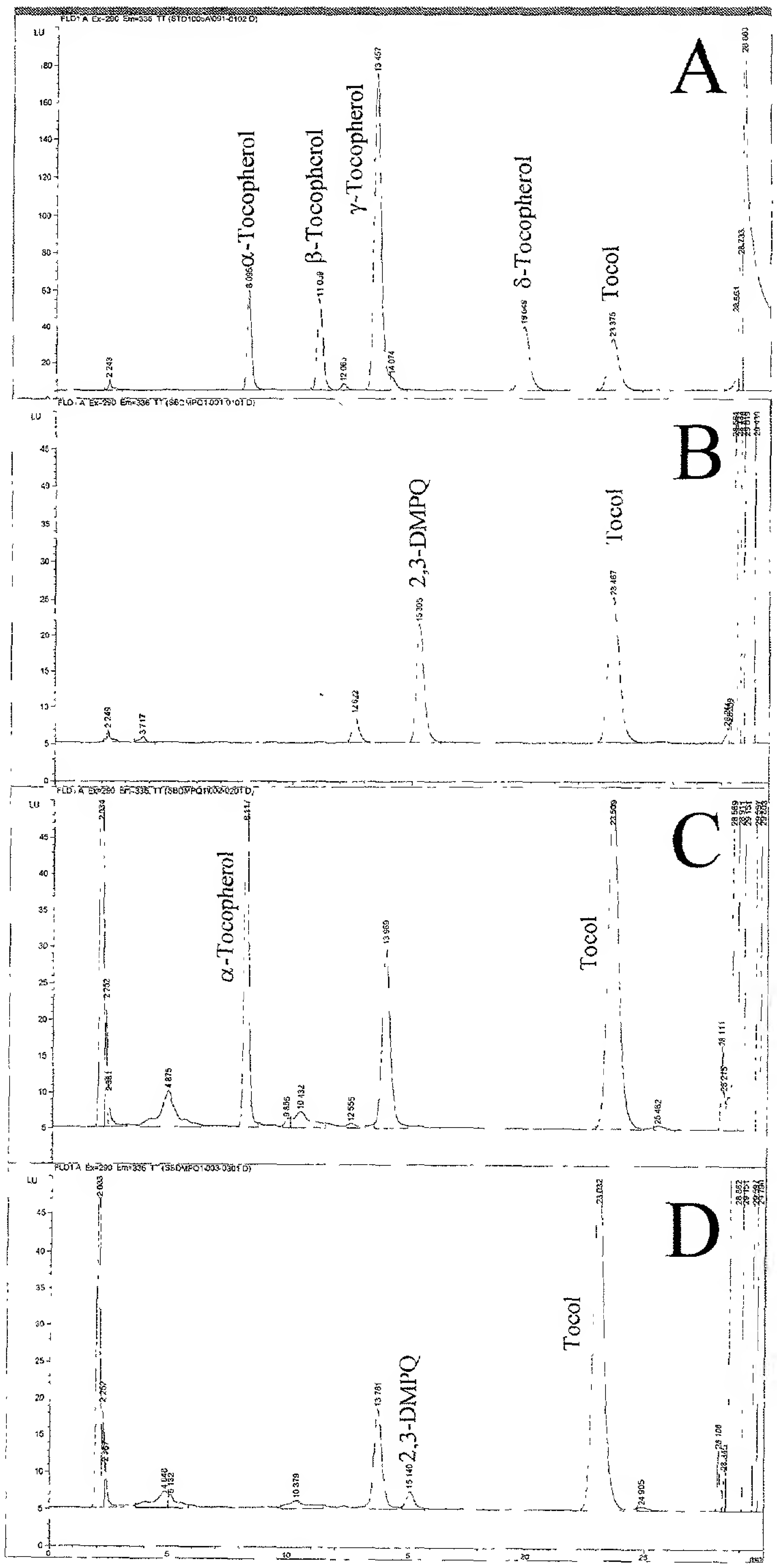

A
α-Tocopherol
β-Tocopherol
γ-Tocopherol
δ-Tocopherol
Tocol
B
2,3-DMPQ
Tocol
C
α-Tocopherol
Tocol
D
Tocol
2,3-DMPQ

Figure 30

```
  Query Sequence: F4D11        AL022537
                 Database: PIR_T04448.atcea.list.fasta
                 Database: PIR_T04448
Plus (+) denotes forward strand, and minus (-) reverse strand.
Asterisks (*) denote bases not shown on pair wise alignments.

                   Alignment 1

                   .    :    .    :    .    :    .    :    .    :    .    :
Query-       12194 CACACGTTCTCGTCCTTTTCTTCTTCCTCTCTGCATTCTTCACAGAGTTTGTCACCACCA
genomic
                   ------------------------------------------------------------
ATCEA4C371+      1                                                            C  est

                   .    :    .    :    .    :    .    :    .    :    .    :first
Met
Query-       12134 ACACCAAACA CACAATTTCACA TCTTTT[redacted]CAT[redacted]TCTT[redacted]TTCTTCTTCCATTATGGA
                   || |||||!|-||||||||||||||||||||||||||||||||||||||||||||||||||
ATCEA4C371+      2 ACCCCAAACATCACAATTTCACATTCTTTTGCATATTTCTTCTTCTTCTTCCATTATGGA

                   .    :    .    :    .    :    .    :    .    :    .    :
Query-       12075 GATACGGAGCTTGATTGTTTCTATGAACCCTAATTTATCTTCCTTTGAGCTCTCTCGCCC
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATCEA4C371+     62 GATACGGAGCTTGATTGTTTCTATGAACCCTAATTTATCTTCCTTTGAGCTCTCTCGCCC

                   .    :    .    :    .    :    .    :    .    :    .    :
Query-       12015 TGTATCTCCTCTCACTCGCTCACTAGTTCCGTTCCGATCGACTAAACTAGTTCCCCGCTC
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATCEA4C371+    122 TGTATCTCCTCTCACTCGCTCACTAGTTCCGTTCCGATCGACTAAACTAGTTCCCCGCTC

                   .    :    .    :    .    :    .    :    .    :    .    :
Query-       11955 CATTTCTAGGGTTTCGGCGTCGATCTCCACCCCGAATAGTGAAACTGACAAGATCTCCGT
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATCEA4C371+    182 CATTTCTAGGGTTTCGGCGTCGATCTCCACCCCGAATAGTGAAACTGACAAGATCTCCGT

                   .    :    .    :    .    :    .    :    .    :    .    :
Query-       11895 TAAACCTGTTTACGTCCCGACGTCTCCCAATCGCGAACTCCGGACTCCTCACAGTGGGTA
                   |||||||||||||||||||||||||||||||||||||||||||||||||||||||||---
ATCEA4C371+    242 TAAACCTGTTTACGTCCCGACGTCTCCCAATCGCGAACTCCGGACTCCTCACAGTGG
                                                             Synecho seq aligns from
here

                   .    :    .    :    .    :    .    :    .    :    .    :
Query-       11835 AATTGATCCATTCCATTCCATTTCTCTTCTCTTGTTTGTTTTATTAAGCTCCAATTTCAG
                   ------------------------------------------------------------
ATCEA4C371+    299

                   ~~ 60 bp removed ~~~

                   .    :    .    :    .    :    .    :    .    :    .    :
Query-       11715 ********************************************************TTTG
                   ------------------------------------------------------------
ATCEA4C371+    299
                                                                           ----
PIR:T04448       1

                   .    :    .    :    .    :    .    :    .    :    .    :
Query-       11655 GTGGCTCACCATTCGACGACTACTTTTGAATTTGAGTTTTTGAAAAATGCAATTTAACAT
                   ------------------------------------------------------------
ATCEA4C371+    299
                   ---------------------------------------------:::::::::::::::
PIR:T04448       1                                              M  Q  F  N  I
                                              arab sequence which is incorrect

                   .    :    .    :    .    :    .    :    .    :    .    :
Query-       11595 CAGAGAGTTTTTTTTTTATGGTTGATAACTTATTGTTTAACTTTTGAAAAATGCAGATA
                   ---------------------------------------------------------|||
ATCEA4C371+    299                                                          ATA
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448       6  R  E  F  F  F  L  W  L  I  T  Y  C  L  T  F  E  K  C  R  Y

                   .    :    .    :    .    :    .    :    .    :    .    :
Query-       11535 CCATTTCGATGGAACACCTCGGAAGTTCTTCGAGGGATGGTATTTCAGGGTTTCCATCCC
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATCEA4C371+    302 CCATTTCGATGGAACACCTCGGAAGTTCTTCGAGGGATGGTATTTCAGGGTTTCCATCCC
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448      26  H  F  D  G  T  P  R  K  F  F  E  G  W  Y  F  R  V  S  I  P

                   .    :    .    :    .    :    .    :    .    :    .    :
Query-       11475 AGAGAAGAGGGAGAGTTTTTGTTTTATGTATTCTGTGGAGAATCCTGCATTTCGGCAGAG
```

```
                  ||||||||||||||||||||||:||||||||||||:|||||||||||||||||||||||:|||||||
ATCEA4C371+    362 AGAGAAGAGGGAGAGTTTTTGTTTTATGTATTCTGTGGAGAATCCTGCATTTCGGCAGAG
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448      46  E  K  R  E  S  F  C  F  M  Y  S  V  E  N  P  A  F  R  Q  S

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       11415 TTTGTCACCATTGGAAGTGGCTCTATATGGACCTAGATTCACTGGTGTTGGAGCTCAGAT
                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATCEA4C371+    422 TTTGTCACCATTGGAAGTGGCTCTATATGGACCTAGATTCACTGGTGTTGGAGCTCAGAT
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448      66  L  S  P  L  E  V  A  L  Y  G  P  R  F  T  G  V  G  A  Q  I

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       11355 TCTTGGCGCTAATGATAAATATTTATGCCAATACGAACAAGACTCTCACAATTTCTGGGG
                   |||||||||||||||||||||||||||||||||||||||||||:||||||||||-----
ATCEA4C371+    482 TCTTGGCGCTAATGATAAATATTTATGCCAATACGAACAAGACTCTCACAATTTC
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448      86  L  G  A  N  D  K  Y  L  C  Q  Y  E  Q  D  S  H  N  F  W  G
ATCEA4C371+        Exon      11538     11301   Confidence: 100 100

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       11295 AGGTAACTCCTTGACCCTTAAAATGCTGTGTCATGACAATAAGAAATCATATCTGAGTCT
                   ---------------------------------------------
ATCEA4C371+    537

                   :.----------------------------------------------------------
PIR:T04448     106  D
PIR:T04448         Exon      11609     11294   Confidence: 100 100

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       11235 TTTCTCTACTTCTAGTACTAATGTTCGTTATTGTTGTTAAAGATCTAAGTCTTATCTGAA
                   ------------------------------------------------------------
PIR:T04448     107

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       11175 TTTTGTTACATTTTGGTTCTGGTGCTTTCTCAACATGAATTTGTATATATGACTTTAAAG
                   ------------------------------------------------------------
PIR:T04448     107

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       11115 ATTGCTTACCTAAAGTTTTTACTCATGCATAGATCGACATGAGCTAGTTTTGGGGAATAC
                   ------------------------------..:::::::::::::::::::::::::::
PIR:T04448     107                                 R  H  E  L  V  L  G  N  T

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       11055 TTTTAGTGCTGTGCCAGGCGCAAAGGCTCCAAACAAGGAGGTTCCACCAGAGGTTCTCAC
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::--------
PIR:T04448     116  F  S  A  V  P  G  A  K  A  P  N  K  E  V  P  P  E
PIR:T04448         Exon      11083     11004   Confidence:  96 100

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       10995 TCCTCCCTTGTTGGTTACTTTGTTATCTGTTAAATAGTTTTCCAATTGTATCCGGATAGT
                   ------------------------------------------------------------
PIR:T04448     133

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       10935 GTTCTACTTCTCCTTGTAGAAAATCTCAAGTTTTTGTTACTCTTGCTATTCTCTTGGATG
                   ------------------------------------------------------------
PIR:T04448     133

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       10875 TTGATTTGTAAAGCATGTCGTTTTATTGTAGGAATTTAACAGAAGAGTGTCCGAAGGGTT
                   ------------------------------::::::::::::::::::::::::::::::
PIR:T04448     133                                E  F  N  R  R  V  S  E  G  F

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       10815 CCAAGCTACTCCATTTTGGCATCAAGGTCACATTTGCGATGATGGCCGGTAATTATATGA
                   :::::::::::::::::::::::::::::::::::::::::::::..------------
PIR:T04448     143  Q  A  T  P  F  W  H  Q  G  H  I  C  D  D  G  R
PIR:T04448         Exon      10844     10768   Confidence: 100 100

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       10755 TTCTATGCACAACAAGAATTCACTATATTATAAATATTGGATATTGAGTATTTTTGTTGA
                   ------------------------------------------------------------
PIR:T04448     159

                        .    :    .    :    .    :    .    :    .    :    .    :
Query-       10695 AAATTTCTGTGTTTAAATCTGACTTGACTTGTTTTGTCAGTACTGACTATGCGGAAACTG
                   ----------------------------------------.::::::::::::::::::::
PIR:T04448     159                                         T  D  Y  A  E  T  V

                        .    :    .    :    .    :    .    :    .    :    .    :
```

Figure 31

2/5

```
Query-       10635 TGAAATCTGCTCGTTGGGAGTATAGTACTCGTCCCGTTTACGGTTGGGGTGATGTTGGGG
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448     166  K  S  A  R  W  E  Y  S  T  R  P  V  Y  G  W  G  D  V  G  A

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-       10575 CCAAACAGAAGTCAACTGCAGGCTGGCCTGCAGCTTTTCCTGTATTTGAGCCTCATTGGC
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448     186  K  Q  K  S  T  A  G  W  P  A  A  F  P  V  F  E  P  H  W  Q

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-       10515 AGATATGCATGGCAGGAGGCCTTTCCACAGGTGTGAGCTTTGCTTGATTGACTTAAAGTT
                   :::::::::::::::::::::::::::::.------------------------------
PIR:T04448     206  I  C  M  A  G  G  L  S  T  G
PIR:T04448         Exon      10655     10486  Confidence:  96 100

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-       10455 AATAAATAGACGGTTAAGTTTACTTGCCTAGTACTAACAGAAAATTAAGAAAGAAACCAC
                   ------------------------------------------------------------
PIR:T04448     216

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-       10395 CCTCTTTCTATCAGCAGAAACTGCTATTGTAGTTCTTATTTTTTCTCTTGTATTTGCAGG
                   -----------------------------------------------------------.
PIR:T04448     216

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-       10335 GTGGATAGAATGGGGCGGTGAAAGGTTTGAGTTTCGGGATGCACCTTCTTATTCAGAGAA
                   .:::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448     216  W  I  F  W  G  G  E  R  F  E  F  R  D  A  P  S  Y  S  E  K

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-       10275 GAATTGGGGTGGAGGCTTCCCAAGAAAATGGTTTTGGGTAAAACATTTCATCCTTTTGCT
                   ::::::::::::::::::::::::::::::::::::------------------------
PIR:T04448     236  N  W  G  G  G  F  P  R  K  W  F  W
PIR:T04448         Exon      10336     10239  Confidence:  96 100

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-       10215 ACATTTCTTGTTGCAGACTTTAGTTAGCTAGTGGACCTGTGTATACACCCACATGTAGTA
                   ------------------------------------------------------------
PIR:T04448     248

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-       10155 TACTTGTTTGATAGCTTTATTTGTCAATGTCTCTTTACAGGTCCAGTGTAATGTCTTTGA
                   ----------------------------------------::::::::::::::::::::
PIR:T04448     248                                          V  Q  C  N  V  F  E

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-       10095 AGGGGCAACTGGAGAAGTTGCTTTAACCGCAGGTGGCGGGTTGAGGCAATTGCCTGGATT
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448     255  G  A  T  G  E  V  A  L  T  A  G  G  G  L  R  Q  L  P  G  L

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-       10035 GACTGAGACCTATGAAAATGCTGCACTGGTATGCACTTATAAGATCTTCTTAAGCAATGA
                   ::::::::::::::::::::::::::::------------------------------------
PIR:T04448     275  T  E  T  Y  E  N  A  A  L
PIR:T04448         Exon      10115     10008  Confidence: 100 100

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9975 CAGTGAGTATTAGAAGGCAGATAGTTTACAAAAGCTCTGGGCCCTTGTAAATCTGCAGGT
                   ----------------------------------------------------------::
PIR:T04448     284                                                            V

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9915 TTGTGTACACTATGATGGAAAAATGTACGAGTTTGTTCCTTGGAATGGTGTTGTTAGATG
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448     285  C  V  H  Y  D  G  K  M  Y  E  F  V  P  W  N  G  V  V  R  W
                       ------------------------------------------------||||||
GSDB:S:495-    532                                                      tagatg

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9855 GGAAATGTCTCCCTGGGG TTATTGGTATATAACTGCAGAGAACGAAAACCATGTGGTAA
                   :::::::::::::::::: :::::::::::::::::::::::::::::::::::----
PIR:T04448     305  E  M  S  P  W  G   Y  W  Y  I  T  A  E  N  E  N  H  V
                   |||||-||||||||||||-||||||||||||||||:|||||| || |||||||||----
GSDB:S:495-    526 ggaaat tctccctgggggttattggtatataactgcagagaNcgNaaaccatgtg
PIR:T04448         Exon      9917      9801   Confidence: 100 100
GSDB:S:495-        Exon      9861      9801   Confidence:  93  93

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9796 ATTTGTTTTACTAGTTTCATTCAGTTTTACTTTTGACATCATATCATTCCCTTATGGCTA
                   ------------------------------------------------------------
```

Figure 31

```
PIR:T04448     323
                   ------------------------------------------------------------
GSDB:S:495-    471

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9736 GATTCCAACACCCGATGAATGTCTTGTGACAGGTGGAACTAGAGGCAAGAACAAATGAAG
                   --------------------------------::::::::::::::::::::::::::::
PIR:T04448     323                                  V  E  L  E  A  R  T  N  E  A
                   --------------------------------|||||||||'||| ||||||||||||||
GSDB:S:495-    471                                 gtggaactagaggcNagaacaaatgaag

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9676 CGGGTACACCTCTGCGTGCTCCTACCACAGAAGTTGGGCTAGCTACGGCTTGCAGAGATA
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448     333  G  T  P  L  R  A  P  T  T  E  V  G  L  A  T  A  C  R  D  S
                   ||||||||||||||||||||||||||||||||||||||||||||||||:|||||||||||
GSDB:S:495-    443 cgggtacacctctgcgtgctcctaccacagaagttgggctagctacggcttgcagagata

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9616 GTTGTTACGGTGAATTGAAGTTGCAGATATGGGAACGGCTATATGATGGAAGTAAAGGCA
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448     353  C  Y  G  E  L  K  L  Q  I  W  E  R  L  Y  D  G  S  K  G  K
                   ||||||||||||||||||||||||||||||:|||||||||||||||||||||||||||||
GSDB:S:495-    383 gttgttacggtgaattgaagttgcagatatgggaacggctatatgatggaagtaaaggca

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9556 AGGTATGTATGCTAATGTGATCCAATCCCTGTAGTTAAAAGTCTTAACAAATCCTAAGGC
                   ::------------------------------::::::::::::::::::::::::::::
PIR:T04448     373                                    L  K  V  L  T  N  P  K  A
                   ||----------------------------------------------------------
GSDB:S:495-    323 ag
PIR:T04448         Exon       9704      9555   Confidence: 100 100
GSDB:S:495-        Exon       9704      9555   Confidence:  98 100

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9496 AGTGAAAGAAGATTATGAACGTTTGTTATGGTTAACAATGATGCAGGTGATATTAGAGAC
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448     382  V  K  E  D  Y  E  R  L  L  W  L  T  M  M  Q  V  I  L  E  T
                   ----------------------------------------------||||||||||||||
GSDB:S:495-    321                                               gtgatattagagac

                       .    .    .    :    .    :    .    :    .    :    .    :
Query-        9436 AAAGAGCTCAATGGCAGCAGTGGAGATAGGAGGAGGACCGTGGTTTGGGACATGGAAAGG
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448     402  K  S  S  M  A  A  V  E  I  G  G  G  P  W  F  G  T  W  K  G
                   ||||||||||||||:||| |||||||||||||||||||||||||||||||||||||||||
GSDB:S:495-    307 aaagagctcaatggcaNcagtggagataggaggaggaccgtggtttgggacatggaaagg

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9376 AGATACGAGCAACACGCCCGAGCTACTAAAACAGGCTCTTCAGGTCCCATTGGATCTTGA
                   ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
PIR:T04448     422  D  T  S  N  T  P  E  L  L  K  Q  A  L  Q  V  P  L  D  L  E
                   |||||||||:||||||||||||||||||||||||||||||||||||||||:||||||||'
GSDB:S:495-    247 agatacgagcaacacgcccgagctactaaaacaggctcttcaggtcccattggatcttga

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9316 AAGCGCCTTAGGTTTGGTCCCTTTCTTCAAGCCACCGGGTCTGTAACATTGATGAGTGTT
(stop)
                   ::::::::::::::::::::::::::::::::::::::::::------------------
PIR:T04448     442  S  A  L  G  L  V  P  F  F  K  P  P  G  L
                   ||||||||||||||||||||||||||||||||||||||||||||||!|||||||||||||
GSDB:S:495-    187 aagcgccttaggtttggtccctttcttcaagccaccgggtctgtaacattgatgagtgtt
PIR:T04448         Exon       9522      9274   Confidence: 100 100

                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9256 TTGTTTGTTGATAGAGACCCATGTGATGAATGAAGCCTTAGTCATGTCATTGCTAGCTTC
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PIR:T04448     456
                   |||||||||||||:||||||||||||||||||||||||||||||||||||||||||||||
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                   ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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                       .    :    .    :    .    :    .    :    .    :    .    :
Query-        9136 GTAAAGTCTAGTTCAATAAAAGCCTTGAGTCGCATAATTTCAATTTCAAATTGCATC
                   |||||||---------------------------------------------------
GSDB:S:495-      7 gtaaagt
GSDB:S:495-        Exon       9450      9130   Confidence:  98 100
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Figure 31
4/5

ATCEA4C37145_1 3063693/emb|CAA18584.1| 4.0e-43 (AL022537) putative protein [Arabidopsis thaliana]

PIR:T04448 sPIR-T04448 shypothetical protein F4D11.30 - Arabidopsis thaliana; g3063693|emb|CAA18584.1 (AL022537) putative protein [Arabidopsis thaliana]_F4D11.30

GSDB:S:4955486|AI995392|AI995392|701673779 A. thaliana, Columbia Col-0, inflorescence-1 Arabidopsis thaliana cDNA clone 701673779, mRNA sequence.

Figure 31
5/5

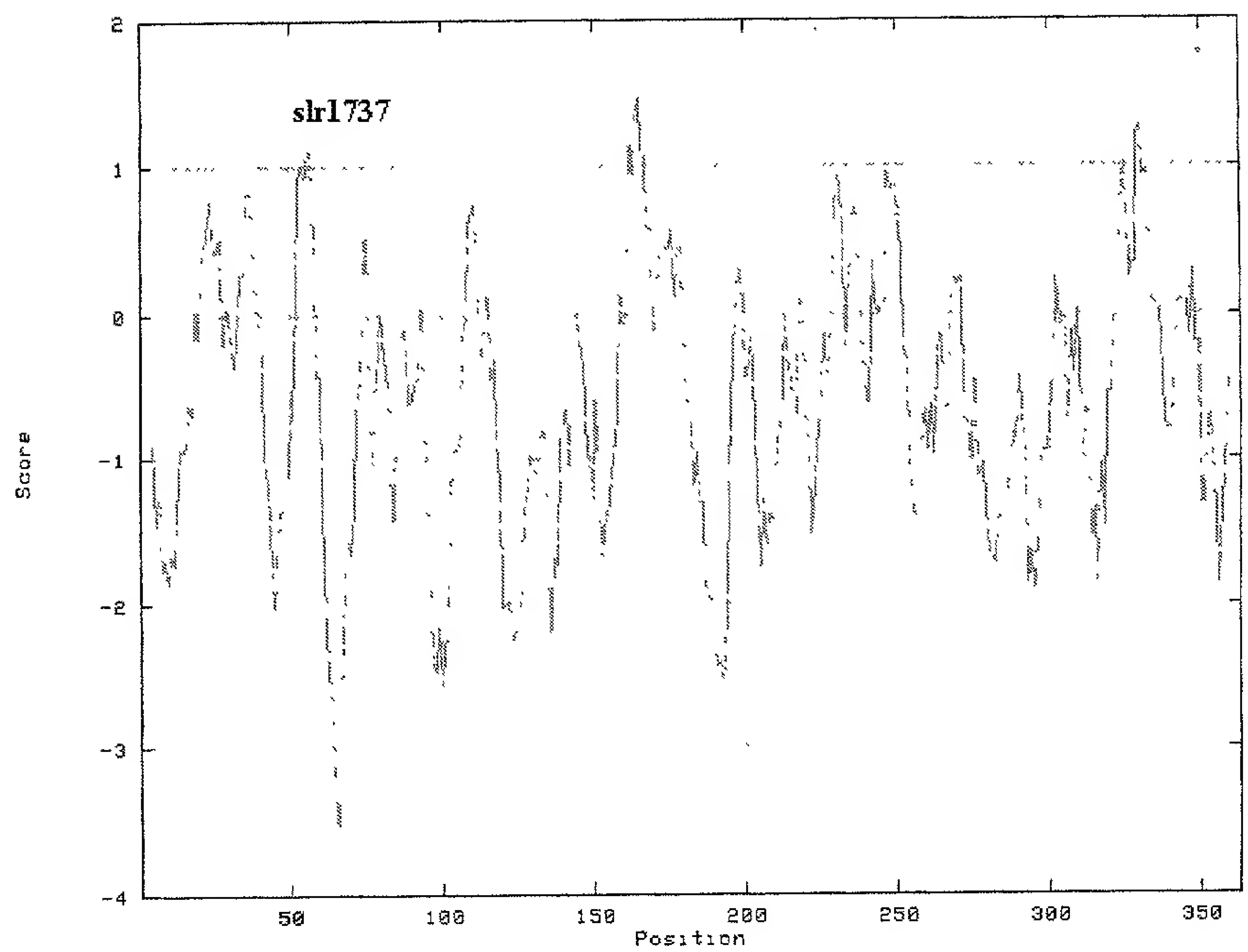


Figure 32

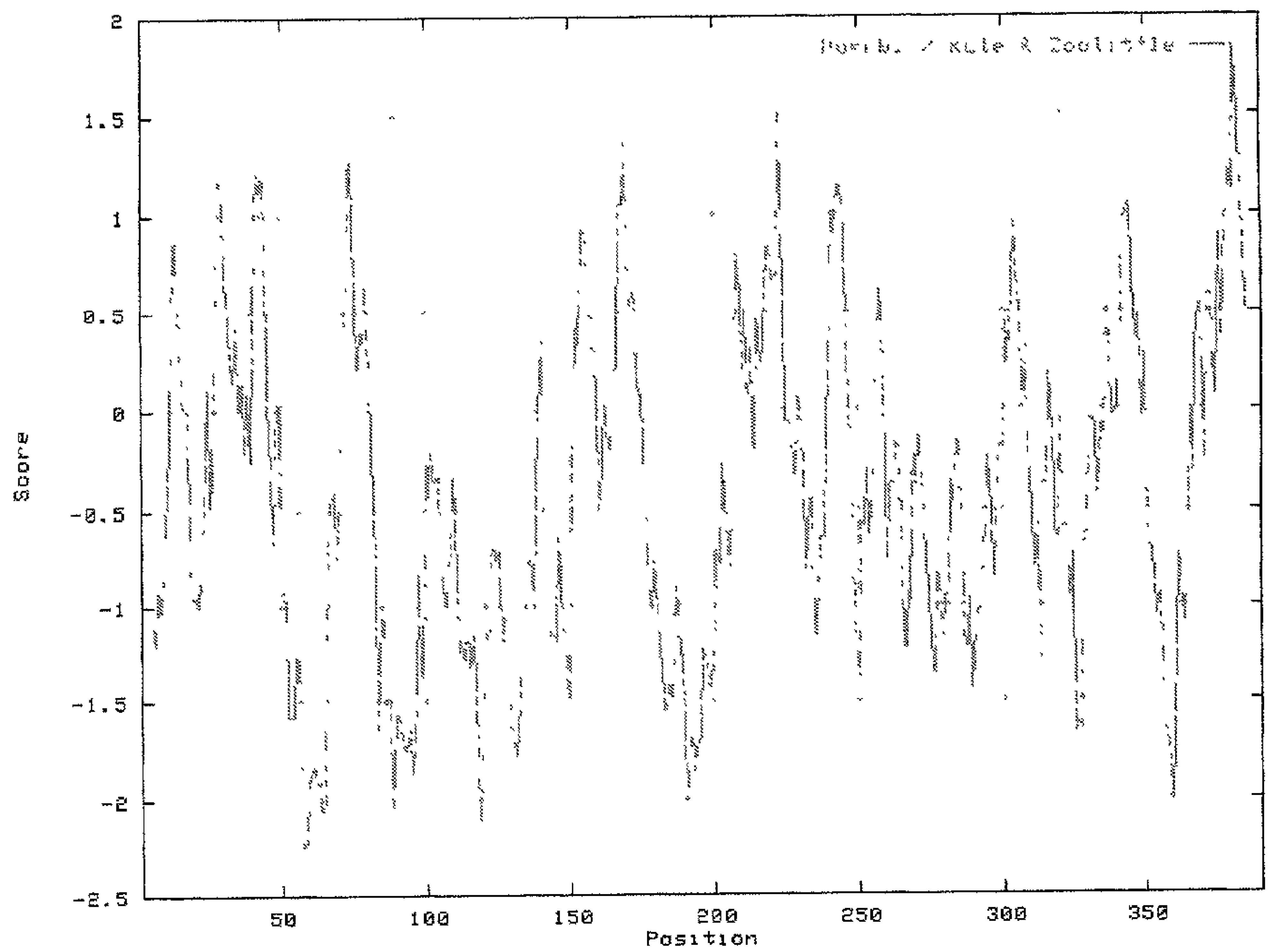
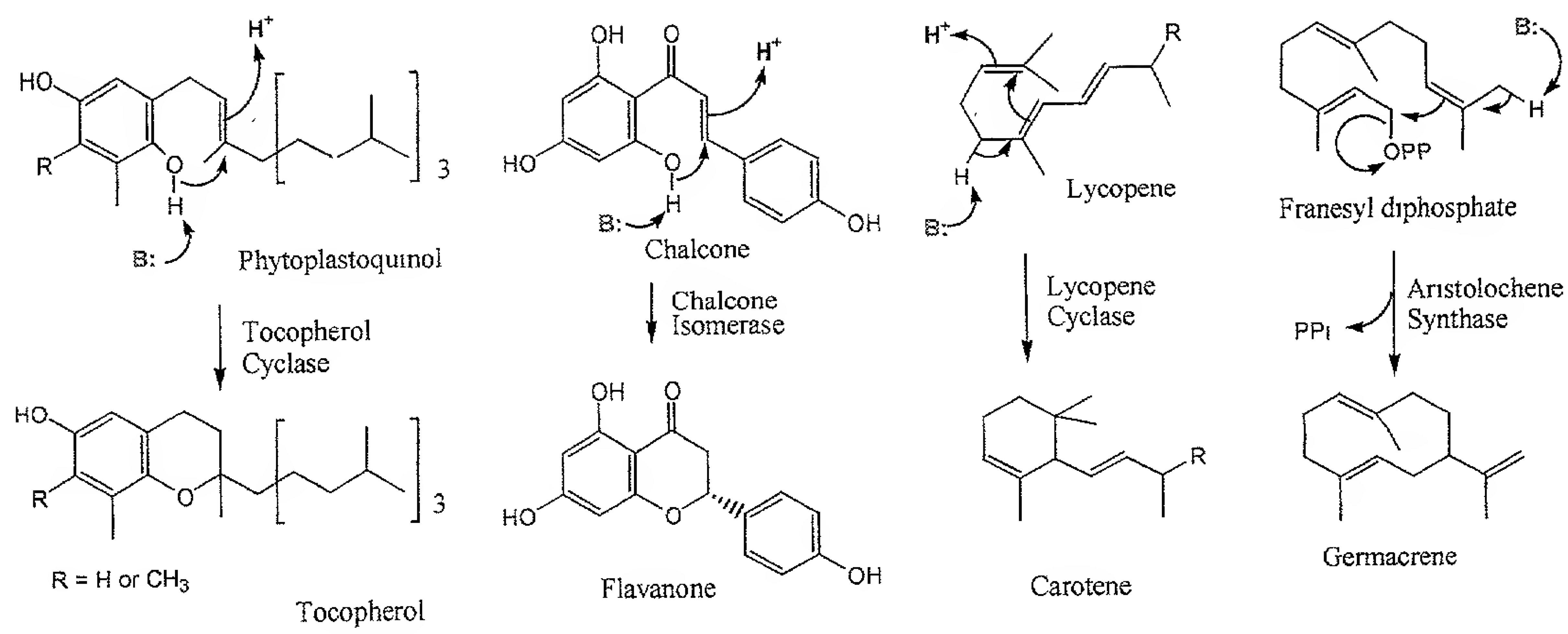


Figure 33



Phytoplastoquinol
Tocopherol Cyclase
R = H or CH3
Tocopherol
Chalcone
Chalcone Isomerase
Flavanone
Lycopene
Lycopene Cyclase
Carotene
Franesyl diphosphate
Aristolochene Synthase
PPi
Germacrene

Figure 34

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slr1737_ARATH_T04448_     KWFWVQCNVFEGATGEVALTAGGGLRQLPGLTETYENAALVCVHYDGKMY
CFI_ARATH_P41088_         VAIWKQLGLYTDCEA-KAV-----EKFLEIFKE---ET------------

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slr1737_ARATH_T04448_     GLATACRDSCYGELKLQIWERLYDGSKGKVILETKSSMAAVEIGGGPWFG
CFI_ARATH_P41088_         --SIIGKNGVSPGTRLSVAERLSQ-----LMMKNKDEKEVSDHSL-----

slr1737_SYNSP_S74814_     ----DWGLTEENLSKKT------------VPF------
slr1737_ARATH_T04448_     TWKGDTSNTPELLKQALQVPLDLESALGLVPFFKPPGL
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Figure 35

SEQUENCE LISTING

<110> Lassner, M
Post-Beittenmiller, D
Savidge, B
Weiss, J

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50 55 60
Tyr Pro Lys His Lys Ser Arg Phe Arg Val Asn Ala Thr Ala Gly Gln
65 70 75 80
Pro Glu Ala Phe Asp Ser Asn Ser Lys Gln Lys Ser Phe Arg Asp Ser
85 90 95
Leu Asp Ala Phe Tyr Arg Phe Ser Arg Pro His Thr Val Ile Gly Thr
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Val Leu Ser Ile Leu Ser Val Ser Phe Leu Ala Val Glu Lys Val Ser
115 120 125
Asp Ile Ser Pro Leu Leu Phe Thr Gly Ile Leu Glu Ala Val Val Ala
130 135 140
Ala Leu Met Met Asn Ile Tyr Ile Val Gly Leu Asn Gln Leu Ser Asp
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Val Glu Ile Asp Lys Val Asn Lys Pro Tyr Leu Pro Leu Ala Ser Gly
165 170 175
Glu Tyr Ser Val Asn Thr Gly Ile Ala Ile Val Ala Ser Phe Ser Ile
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Met Ser Phe Trp Leu Gly Trp Ile Val Gly Ser Trp Pro Leu Phe Trp

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                245                 250                 255
Gln Thr His Val Phe Gly Arg Pro Ile Leu Phe Thr Arg Pro Leu Ile
            260                 265                 270
Phe Ala Thr Ala Phe Met Ser Phe Phe Ser Val Val Ile Ala Leu Phe
        275                 280                 285
Lys Asp Ile Pro Asp Ile Glu Gly Asp Lys Ile Phe Gly Ile Arg Ser
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Phe Ser Val Thr Leu Gly Gln Lys Arg Val Phe Trp Thr Cys Val Thr
305                 310                 315                 320
Leu Leu Gln Met Ala Tyr Ala Val Ala Ile Leu Val Gly Ala Thr Ser
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Pro Phe Ile Trp Ser Lys Val Ile Ser Val Val Gly His Val Ile Leu
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Ala Thr Thr Leu Trp Ala Arg Ala Lys Ser Val Asp Leu Ser Ser Lys
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actacccatt acacaaatcc tttcactaag tgttatcctt catggaatga taattaccaa     180
gtatggagta aaggaagaga attgcatcag gagaagtttt ttggtgttgg ttggaattac     240
agattaattt gtggaatgtc gtcgtcttct tcggttttgg agggaaagcc gaagaaagat     300
gataaggaga agagtgatgg tgttgttgtt aagaaagctt cttggataga tttgtattta     360
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Thr Lys Cys Tyr Pro Ser Trp Asn Asp Asn Tyr Gln Val Trp Ser Lys
    50                  55                  60
Gly Arg Glu Leu His Gln Glu Lys Phe Phe Gly Val Gly Trp Asn Tyr
65                  70                  75                  80
Arg Leu Ile Cys Gly Met Ser Ser Ser Ser Ser Val Leu Glu Gly Lys
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Pro Lys Lys Asp Asp Lys Glu Lys Ser Asp Gly Val Val Val Lys Lys
            100                 105                 110
Ala Ser Trp Ile Asp Leu Tyr Leu Pro Glu Glu Val Arg Gly Tyr Ala
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Lys Leu Ala Arg Leu Asp Lys Pro Ile Gly Thr Trp Leu Leu Ala Trp
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Pro Cys Met Trp Ser Ile Ala Leu Ala Ala Asp Pro Gly Ser Leu Pro
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Ser Phe Lys Tyr Met Ala Leu Phe Gly Cys Gly Ala Leu Leu Leu Arg
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Pro Phe Gln Gly Ile Gly Phe Leu Gly Leu Gln Leu Leu Leu Gly Leu
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Gly Ile Leu Leu Gln Leu Asn Asn Tyr Ser Arg Val Leu Gly Ala Ser
225                 230                 235                 240
Ser Leu Leu Leu Val Phe Ser Tyr Pro Leu Met Lys Arg Phe Thr Phe
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Trp Pro Gln Ala Phe Leu Gly Leu Thr Ile Asn Trp Gly Ala Leu Leu
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Gly Trp Thr Ala Val Lys Gly Ser Ile Ala Pro Ser Ile Val Leu Pro
        275                 280                 285
Leu Tyr Leu Ser Gly Val Cys Trp Thr Leu Val Tyr Asp Thr Ile Tyr
    290                 295                 300
Ala His Gln Asp Lys Glu Asp Asp Val Lys Val Gly Val Lys Ser Thr
305                 310                 315                 320
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Thr Ala Ser Ile Gly Phe Leu Ala Leu Ser Gly Phe Ser Ala Asp Leu
            340                 345                 350
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        355                 360                 365
Trp Gln Ile Gly Thr Ala Asp Leu Ser Ser Gly Ala Asp Cys Ser Arg
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aatcgagttt ttgccactgc tactgccgcc gctacagcta cagctaccac cggtgagatt     240
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Ala Lys Leu Gly Ile Thr Gly Val Arg Ser Asp Ala Asn Arg Val Phe
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115 120 125

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165 170 175
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Leu Pro Ala Ala Leu Tyr Phe Trp Gln Ile Pro His Phe Met Ala Leu
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325 330 335
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        35   40   45

Glu Ser Thr Asp Ile Val Thr Ser Glu Leu Arg Val Arg Gln Arg Gly
    50   55   60

Ile Ala Glu Ile Thr Glu Met Ile His Val Ala Ser Leu Leu His Asp
65   70   75   80

Asp Val Leu Asp Asp Ala Asp Thr Arg Arg Gly Val Gly Ser Leu Asn
                85   90   95

Val Val Met Gly Asn Lys Val Val Ala Leu Leu Ala Thr Ala Val Glu
            100   105   110

His Leu Val Thr Gly Glu Thr Met Glu Ile Thr Ser Ser Thr Glu Gln
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Arg Tyr Ser Met Asp Tyr Tyr Met Gln Lys Thr Tyr Tyr Lys Thr Ala
    130   135   140

Ser Leu Ile Ser Asn Ser Cys Lys Ala Val Ala Val Leu Thr Gly Gln
145   150   155   160

Thr Ala Glu Val Ala Val Leu Ala Phe Glu Tyr Gly Arg Asn Leu Gly
                165   170   175

Leu Ala Phe Gln Leu Ile Asp Asp Ile Leu Asp Phe Thr Gly Thr Ser
            180   185   190

Ala Ser Leu Gly Lys Gly Ser Leu Ser Asp Ile Arg His Gly Val Ile
        195   200   205

Thr Ala Pro Ile Leu Phe Ala Met Glu Glu Phe Pro Gln Leu Arg Glu
    210   215   220

Val Val Asp Gln Val Glu Lys Asp Pro Arg Asn Val Asp Ile Ala Leu
225   230   235   240

Glu Tyr Leu Gly Lys Ser Lys Gly Ile Gln Arg Ala Arg Glu Leu Ala
                245   250   255

Met Glu His Ala Asn Leu Ala Ala Ala Ala Ile Gly Ser Leu Pro Glu
            260   265   270

Thr Asp Asn Glu Asp Val Lys Arg Ser Arg Arg Ala Leu Ile Asp Leu
        275   280   285

Thr His Arg Val Ile Thr Arg Asn Lys
    290   295

<210> 10

<211> 561

<212> DNA
<213> Arabidopsis sp

<400> 10
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acgtcgtcga tgaaagcgat ttgcgccgcg gccgcgaaag cgcgcataag gttttcggca 180
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gcgaagtgat gcagctcggc accgcgcgca atcttgaaac caatatgagc cagtatctcg 360
atgtgatcag cgcgaagacc gccgcgctct ttgccgccgc ctgcgaaatc ggcccggtga 420
tggcgaacgc gaaggcggaa gatgctgccg cgatgtgcga atacggcatg aatctcggta 480
tcgccttcca gatcatcgac gaccttctcg attacggcac cggcggccac gccgagcttg 540
gcaagaacac gggcgacgat t 561

<210> 11
<211> 966
<212> DNA
<213> Arabidopsis sp

<400> 11
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aggcaacggg gtattgctga aatcactgaa atgatacacg tcgcaagtct actgcacgat 240
gatgtcttgg atgatgccga tacaaggcgt ggtgttggtt ccttaaatgt tgtaatgggt 300
aacaagatgt cggtattagc aggagacttc ttgctctccc gggcttgtgg ggctctcgct 360
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aagggatcgt tgtcagatat tcgccatgga gtcataacag ccccaatcct ctttgccatg 720
gaagagtttc ctcaactacg cgaagttgtt gatcaagttg aaaaagatcc taggaatgtt 780
gacattgctt tagagtatct tgggaagagc aagggaatac agagggcaag agaattagcc 840
atggaacatg cgaatctagc agcagctgca atcgggtctc tacctgaaac agacaatgaa 900
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aagtga 966

<210> 12
<211> 321

<212> PRT
<213> Arabidopsis sp

<400> 12

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35 40 45
Glu Ser Thr Asp Ile Val Thr Ser Glu Leu Arg Val Arg Gln Arg Gly
50 55 60
Ile Ala Glu Ile Thr Glu Met Ile His Val Ala Ser Leu Leu His Asp
65 70 75 80
Asp Val Leu Asp Asp Ala Asp Thr Arg Arg Gly Val Gly Ser Leu Asn
85 90 95
Val Val Met Gly Asn Lys Met Ser Val Leu Ala Gly Asp Phe Leu Leu
100 105 110
Ser Arg Ala Cys Gly Ala Leu Ala Ala Leu Lys Asn Thr Glu Val Val
115 120 125
Ala Leu Leu Ala Thr Ala Val Glu His Leu Val Thr Gly Glu Thr Met
130 135 140
Glu Ile Thr Ser Ser Thr Glu Gln Arg Tyr Ser Met Asp Tyr Tyr Met
145 150 155 160
Gln Lys Thr Tyr Tyr Lys Thr Ala Ser Leu Ile Ser Asn Ser Cys Lys
165 170 175
Ala Val Ala Val Leu Thr Gly Gln Thr Ala Glu Val Ala Val Leu Ala
180 185 190
Phe Glu Tyr Gly Arg Asn Leu Gly Leu Ala Phe Gln Leu Ile Asp Asp
195 200 205
Ile Leu Asp Phe Thr Gly Thr Ser Ala Ser Leu Gly Lys Gly Ser Leu
210 215 220
Ser Asp Ile Arg His Gly Val Ile Thr Ala Pro Ile Leu Phe Ala Met
225 230 235 240
Glu Glu Phe Pro Gln Leu Arg Glu Val Val Asp Gln Val Glu Lys Asp
245 250 255
Pro Arg Asn Val Asp Ile Ala Leu Glu Tyr Leu Gly Lys Ser Lys Gly
260 265 270
Ile Gln Arg Ala Arg Glu Leu Ala Met Glu His Ala Asn Leu Ala Ala
275 280 285
Ala Ala Ile Gly Ser Leu Pro Glu Thr Asp Asn Glu Asp Val Lys Arg

290 295 300

Ser Arg Arg Ala Leu Ile Asp Leu Thr His Arg Val Ile Thr Arg Asn

305 310 315 320

Lys

<210> 13
<211> 621
<212> DNA
<213> Arabidopsis sp

<400> 13
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ggctcattct ctagcgcgaa gatcactggc gccgttatgt tacctttggc taagtcatta 180
gctgcaggct tacctaactg ctctgtggac tgagtgaagt ccagaatgtc atcaactact 240
tgaaaagata aaccgagatt cttcccgaac tgatacattt gctctgcgac cttgctttcg 300
actttactga aaattgctgc tcctttggtg cttgcagcta ctaatgaagc tgtcttgtag 360
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tttatctcac cgcttgcaaa atctttgatc acctgcaaaa agataaatca agattcagac 480
caaatgttct ttgtattgag tagcttcatc taatctcaga aaggaatatt acctgactta 540
tgagcttaat gacttcaagg ttttcgagat ttgtaagtac catgatgctt gagcaacatg 600
aaatccccag ctaatacagc t 621

<210> 14
<211> 741
<212> DNA
<213> Arabidopsis sp

<400> 14
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gtagcggtgc tagctggaga tttcatgttt gctcaagcgt catggtactt agcaaatctc 180
gagaatcttg aagttattaa gctcatcagt caggtactta gttactctta cattgttttt 240
ctatgaggtt gagctatgaa tctcatttcg ttgaataatg ctgtgcctca aacttttttt 300
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ttgactgcga caccaagctc gacgagtact tactcaaaag tttctacaag acagcctctt 420
tagtggctgc gagcaccaaa ggagctgcca ttttcagcag agttgagcct gatgtgacag 480
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tggatttcac tcagtcgaca gagcagctcg ggaagccagc agggagtgat ttggctaaag 600
gtaacttaac agcacctgtg attttcgctc tggagaggga gccaaggcta agagagatca 660

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<210> 15
<211> 1087
<212> DNA
<213> Arabidopsis sp

<400> 15

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ttcaaccaga gggaaaaagc aacgataaca actctgcttt tgatttcaag ctgtatatga     180
tccgcaaagc cgagtctgta aatgcggctc tcgacgtttc cgtaccgctt ctgaaacccc     240
ttacgatcca agaagcggtc aggtactctt tgctagccgg cggaaaacgt gtgaggcctc     300
tgctctgcat tgccgcttgt gagcttgtgg ggggcgacga ggctactgcc atgtcagccg     360
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gccagggcca tagggactac agggctagtt gctggacaaa tgatagacct agccagcgaa     720
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gcggcattgt tggaggcagc ggcagtttta ggggttataa tgggaggtgg aacagaggaa     840
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gacattctcg acgtaacaaa atctactgag gaattgggta agacagccgg aaaagacgta     960
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cctctgg                                                              1087
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<210> 16
<211> 1164
<212> DNA
<213> Arabidopsis sp

<400> 16

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ggtttctcga cgttgatcta cgaatcaccc gggcggagat ttgttgtgcg tgcggcggag     180
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attaaccagc ttctcggtat caaaggagca tctcaagaaa ctaataaatg gaagattcgt     300
cttcagctta caaaaccagt cacttggcct ccactggttt ggggagtcgt ctgtggtgct     360
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gctgcttcag ggaactttca ttggacccca gaggatgttg ctaagtcgat tctttgcatg 420
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gatatcgacg caattaatga gccatatcgt ccaattccat ctggagcaat atcagagcca 540
gaggttatta cacaagtctg ggtgctatta ttgggaggtc ttggtattgc tggaatatta 600
gatgtgtggg cagggcatac cactcccact gtcttctatc ttgctttggg aggatcattg 660
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tttgcacttg gagcaagcta tattagtttg ccatggtggg ctggccaagc attgtttggc 780
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<210> 17
<211> 387
<212> PRT
<213> Arabidopsis sp

<400> 17
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            20   25   30
Val Glu Phe Thr Arg Arg Arg Ser Gly Phe Ser Thr Leu Ile Tyr Glu
        35   40   45
Ser Pro Gly Arg Arg Phe Val Val Arg Ala Ala Glu Thr Asp Thr Asp
    50   55   60
Lys Val Lys Ser Gln Thr Pro Asp Lys Ala Pro Ala Gly Gly Ser Ser
65   70   75   80
Ile Asn Gln Leu Leu Gly Ile Lys Gly Ala Ser Gln Glu Thr Asn Lys
                85   90   95
Trp Lys Ile Arg Leu Gln Leu Thr Lys Pro Val Thr Trp Pro Pro Leu
            100   105   110
Val Trp Gly Val Val Cys Gly Ala Ala Ala Ser Gly Asn Phe His Trp
        115   120   125
Thr Pro Glu Asp Val Ala Lys Ser Ile Leu Cys Met Met Met Ser Gly
    130   135   140
Pro Cys Leu Thr Gly Tyr Thr Gln Thr Ile Asn Asp Trp Tyr Asp Arg
145   150   155   160

Asp Ile Asp Ala Ile Asn Glu Pro Tyr Arg Pro Ile Pro Ser Gly Ala
165 170 175
Ile Ser Glu Pro Glu Val Ile Thr Gln Val Trp Val Leu Leu Leu Gly
180 185 190
Gly Leu Gly Ile Ala Gly Ile Leu Asp Val Trp Ala Gly His Thr Thr
195 200 205
Pro Thr Val Phe Tyr Leu Ala Leu Gly Gly Ser Leu Leu Ser Tyr Ile
210 215 220
Tyr Ser Ala Pro Pro Leu Lys Leu Lys Gln Asn Gly Trp Val Gly Asn
225 230 235 240
Phe Ala Leu Gly Ala Ser Tyr Ile Ser Leu Pro Trp Trp Ala Gly Gln
245 250 255
Ala Leu Phe Gly Thr Leu Thr Pro Asp Val Val Val Leu Thr Leu Leu
260 265 270
Tyr Ser Ile Ala Gly Leu Gly Ile Ala Ile Val Asn Asp Phe Lys Ser
275 280 285
Val Glu Gly Asp Arg Ala Leu Gly Leu Gln Ser Leu Pro Val Ala Phe
290 295 300
Gly Thr Glu Thr Ala Lys Trp Ile Cys Val Gly Ala Ile Asp Ile Thr
305 310 315 320
Gln Leu Ser Val Ala Gly Tyr Leu Leu Ala Ser Gly Lys Pro Tyr Tyr
325 330 335
Ala Leu Ala Leu Val Ala Leu Ile Ile Pro Gln Ile Val Phe Gln Phe
340 345 350
Lys Tyr Phe Leu Lys Asp Pro Val Lys Tyr Asp Val Lys Tyr Gln Ala
355 360 365
Ser Ala Gln Pro Phe Leu Val Leu Gly Ile Phe Val Thr Ala Leu Ala
370 375 380
Ser Gln His
385

<210> 18
<211> 981
<212> DNA
<213> Arabidopsis sp

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gggtcgttga acttcgatct gaggacgtat tggacgactc tgatcaccga gatcaaccag 180
aagctggatg aggccatacc ggtcaagcac cctgcgggga tctacgaggc tatgagatac 240

tctgtactcg cacaaggcgc caagcgtgcc cctcctgtga tgtgtgtggc ggcctgcgag 300
ctcttcggtg gcgatcgcct cgccgctttc cccaccgcct gtgccctaga aatggtgcac 360
gcggcttcgt tgatacacga cgacctcccc tgtatggacg acgatcctgt gcgcagagga 420
aagccatcta accacactgt ctacggctct ggcatggcca ttctcgccgg tgacgccctc 480
ttcccactcg ccttccagca cattgtctcc cacacgcctc ctgaccttgt tccccgagcc 540
accatcctca gactcatcac tgagattgcc cgcactgtcg gctccactgg tatggctgca 600
ggccagtacg tcgaccttga aggaggtccc tttcctcttt cctttgttca ggagaagaaa 660
ttcggagcca tgggtgaatg ctctgccgtg tgcggtggcc tattgggcgg tgccactgag 720
gatgagctcc agagtctccg aaggtacggg agagccgtcg ggatgctgta tcaggtggtc 780
gatgacatca ccgaggacaa gaagaagagc tatgatggtg gagcagagaa gggaatgatg 840
gaaatggcgg aagagctcaa ggagaaggcg aagaaggagc ttcaagtgtt tgacaacaag 900
tatggaggag gagacacact tgttcctctc tacaccttcg ttgactacgc tgctcatcga 960
catttcttc ttcccctctg a 981

<210> 19
<211> 245
<212> DNA
<213> GLycine sp

<400> 19
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tcttgcactt gcttgggtac catgatggtt gctgcatctg ctaactcttt gaatcaggtg 120
tttgagatca ataatgatgc taaaatgaag agaacaagtc gcaggccact accctcagga 180
cgcatcacaa tacctcatgc agttggctgg gcatcctctg ttggattagc tggtacggct 240
ctact 245

<210> 20
<211> 253
<212> DNA
<213> Glycine sp

<400> 20
attggctttc caagatcatt gggttttctt gttgcattca tgaccttcta ctccttgggt 60
ttggcattgt ccaaggatat acctgacgtt gaaggagata aagagcacgg cattgattct 120
tttgcagtac gtctaggtca gaaacgggca ttttggattt gcgtttcctt ttttgaaatg 180
gctttcggag ttggtatcct ggccggagca tcatgctcac acttttggac taaaattttc 240
acgggtatgg gaa 253

<210> 21
<211> 275
<212> DNA

<213> Glycine sp

<400> 21
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gcattatcct ttttgaaatg gcttttggag ttgccctctt ggcaggagca acatcttctt 180
acctttggat taaaattgtc acgggtctgg gacatgctat tcttgcttca attctcttgt 240
accaagccaa atctatatac ttgagcaaca aagtt 275

<210> 22
<211> 299
<212> DNA
<213> Glycine sp

<220>
<221> misc_feature
<222> (1)...(299)
<223> n = A,T,C or G

<400> 22
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tctttgtggg tatggcattg gcaaaggata tacctanctg ttgaaggaga taaaatatat 180
ggcattgata cttttgcaat acgtataggt caaaaacaag tattttggat ttgtattttc 240
ctttttgaaa ggctttcgga gtttccctag tggcaggagc aacatcttct agccttggt 299

<210> 23
<211> 767
<212> DNA
<213> Glycine sp

<400> 23
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tcctttgaaa ctggtgtcac tattgttgca tctttttcaa ttctgagttt ttggcttggc 180
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<210> 24
<211> 255
<212> PRT
<213> Glycine sp

<400> 24

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            20                  25                  30
Leu Pro Leu Ala Ser Gly Glu Tyr Ser Phe Glu Thr Gly Val Thr Ile
        35                  40                  45
Val Ala Ser Phe Ser Ile Leu Ser Phe Trp Leu Gly Trp Val Val Gly
    50                  55                  60
Ser Trp Pro Leu Phe Trp Ala Leu Phe Val Ser Phe Val Leu Gly Thr
65                  70                  75                  80
Ala Tyr Ser Ile Asn Val Pro Leu Leu Arg Trp Lys Arg Phe Ala Val
                85                  90                  95
Leu Ala Ala Met Cys Ile Leu Ala Val Arg Ala Val Ile Val Gln Leu
            100                 105                 110
Ala Phe Phe Leu His Met Gln Thr His Val Tyr Lys Arg Pro Pro Val
        115                 120                 125
Phe Ser Arg Pro Leu Ile Phe Ala Thr Ala Phe Met Ser Phe Phe Ser
    130                 135                 140
Val Val Ile Ala Leu Phe Lys Asp Ile Pro Asp Ile Glu Gly Asp Lys
145                 150                 155                 160
Val Phe Gly Ile Gln Ser Phe Ser Val Cys Leu Gly Gln Lys Pro Val
                165                 170                 175
Phe Trp Thr Cys Val Thr Leu Leu Glu Ile Ala Tyr Gly Val Ala Leu
            180                 185                 190
Leu Val Gly Ala Ala Ser Pro Cys Leu Trp Ser Lys Ile Phe Thr Gly
        195                 200                 205
Leu Gly His Ala Val Leu Ala Ser Ile Leu Trp Phe His Ala Lys Ser
    210                 215                 220
Val Asp Leu Lys Ser Lys Ala Ser Ile Thr Ser Phe Tyr Met Phe Ile
225                 230                 235                 240
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Trp Lys Leu Phe Tyr Ala Glu Tyr Leu Leu Ile Pro Phe Val Arg
245 250 255

<210> 25
<211> 360
<212> DNA
<213> Zea sp

<220>
<221> misc_feature
<222> (1)...(360)
<223> n = A,T,C or G

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ggaaagcata gaccctgcaa atcatccttc cattgtatac agctggtatt tgttggacgc 180
tggtgtatga tactatatat gcgcatcagg tgtttcgcta tccctacttt catattaatc 240
cttgatgaag tggccatttc atgttgtcgc ggtggtctta tacttgcata tctccatgca 300
tctcaggaca aagangatga cctgaaagta ggagtccaag tccacagctt aagatttggg 360

<210> 26
<211> 299
<212> DNA
<213> Zea sp

<220>
<221> misc_feature
<222> (1)...(299)
<223> n = A,T,C or G

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atgtgggcta caagtgttgg agttgcagga acagctttgt tggcctggaa ggctaatggc 180
ttggcagctg ggcttgcagc ttctaatctt gttctgtatg catttgtgta tacgccgttg 240
aagcaaatac accctgttaa tacatgggtt ggggcagtcg ttggtgccat cccaccact 299

<210> 27
<211> 255
<212> DNA

<213> Zea sp

<220>
<221> misc_feature
<222> (1)...(255)
<223> n = A,T,C or G

<400> 27
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tgcttcggca gcttagcact cagtggttac aatgctgacc ttggttggtg tttagtgtga 180
tgcttgagcg aagaatggta tngtttttac ttgatattga ctccagacct gaaatcatgt 240
tggacagggt ggccc 255

<210> 28
<211> 257
<212> DNA
<213> Zea sp

<400> 28
attgaagggg ataggactct ggggcttcag tcacttcctg ttgcttttgg gatggaaact 60
gcaaaatgga tttgtgttgg agcaattgat atcactcaat tatctgttgc aggttaccta 120
ttgagcaccg gtaagctgta ttatgccctg gtgttgcttg ggctaacaat tcctcaggtg 180
ttctttcagt tccagtactt cctgaaggac cctgtgaagt atgatgtcaa atatcaggca 240
agcgcacaac cattctt 257

<210> 29
<211> 368
<212> DNA
<213> Zea sp

<400> 29
atccagttgc aaataataat ggcgttcttc tctgttgtaa tagcactatt caaggatata 60
cctgacatcg aaggggaccg catattcggg atccgatcct tcagcgtccg gttagggcaa 120
aagaaggtct tttggatctg cgttggcttg cttgagatgg cctacagcgt tgcgatactg 180
atgggagcta cctcttcctg tttgtggagc aaaacagcaa ccatcgctgg ccattccata 240
cttgccgcga tcctatggag ctgcgcgcga tcggtggact tgacgagcaa agccgcaata 300
acgtccttct acatgttcat ctggaagctg ttctacgcgg agtacctgct catccctctg 360
gtgcggtg 368

<210> 30

<211> 122
<212> PRT
<213> Zea sp

<400> 30
Ile Gln Leu Gln Ile Ile Met Ala Phe Phe Ser Val Val Ile Ala Leu
1   5   10   15
Phe Lys Asp Ile Pro Asp Ile Glu Gly Asp Arg Ile Phe Gly Ile Arg
            20   25   30
Ser Phe Ser Val Arg Leu Gly Gln Lys Lys Val Phe Trp Ile Cys Val
        35   40   45
Gly Leu Leu Glu Met Ala Tyr Ser Val Ala Ile Leu Met Gly Ala Thr
    50   55   60
Ser Ser Cys Leu Trp Ser Lys Thr Ala Thr Ile Ala Gly His Ser Ile
65   70   75   80
Leu Ala Ala Ile Leu Trp Ser Cys Ala Arg Ser Val Asp Leu Thr Ser
                85   90   95
Lys Ala Ala Ile Thr Ser Phe Tyr Met Phe Ile Trp Lys Leu Phe Tyr
            100   105   110
Ala Glu Tyr Leu Leu Ile Pro Leu Val Arg
        115   120

<210> 31
<211> 278
<212> DNA
<213> Zea sp

<400> 31
tattcagcac cacctctcaa gctcaagcag aatggatgga ttgggaactt cgctctgggt 60
gcgagttaca tcagcttgcc ctggtgggct ggccaggcgt tatttggaac tcttacacca 120
gatatcattg tcttgactac tttgtacagc atagctgggc tagggattgc tattgtaaat 180
gatttcaaga gtattgaagg ggataggact ctggggcttc agtcacttcc tgttgctttt 240
gggatggaaa ctgcaaaatg gatttgtgtt ggagcaat 278

<210> 32
<211> 292
<212> PRT
<213> Synechocystis sp

<400> 32
Met Val Ala Gln Thr Pro Ser Ser Pro Pro Leu Trp Leu Thr Ile Ile

```
1               5                   10                  15
Tyr Leu Leu Arg Trp His Lys Pro Ala Gly Arg Leu Ile Leu Met Ile
            20                  25                  30
Pro Ala Leu Trp Ala Val Cys Leu Ala Ala Gln Gly Leu Pro Pro Leu
        35                  40                  45
Pro Leu Leu Gly Thr Ile Ala Leu Gly Thr Leu Ala Thr Ser Gly Leu
    50                  55                  60
Gly Cys Val Val Asn Asp Leu Trp Asp Arg Asp Ile Asp Pro Gln Val
65                  70                  75                  80
Glu Arg Thr Lys Gln Arg Pro Leu Ala Ala Arg Ala Leu Ser Val Gln
                85                  90                  95
Val Gly Ile Gly Val Ala Leu Val Ala Leu Leu Cys Ala Ala Gly Leu
            100                 105                 110
Ala Phe Tyr Leu Thr Pro Leu Ser Phe Trp Leu Cys Val Ala Ala Val
        115                 120                 125
Pro Val Ile Val Ala Tyr Pro Gly Ala Lys Arg Val Phe Pro Val Pro
    130                 135                 140
Gln Leu Val Leu Ser Ile Ala Trp Gly Phe Ala Val Leu Ile Ser Trp
145                 150                 155                 160
Ser Ala Val Thr Gly Asp Leu Thr Asp Ala Thr Trp Val Leu Trp Gly
                165                 170                 175
Ala Thr Val Phe Trp Thr Leu Gly Phe Asp Thr Val Tyr Ala Met Ala
            180                 185                 190
Asp Arg Glu Asp Asp Arg Arg Ile Gly Val Asn Ser Ser Ala Leu Phe
        195                 200                 205
Phe Gly Gln Tyr Val Gly Glu Ala Val Gly Ile Phe Phe Ala Leu Thr
    210                 215                 220
Ile Gly Cys Leu Phe Tyr Leu Gly Met Ile Leu Met Leu Asn Pro Leu
225                 230                 235                 240
Tyr Trp Leu Ser Leu Ala Ile Ala Ile Val Gly Trp Val Ile Gln Tyr
                245                 250                 255
Ile Gln Leu Ser Ala Pro Thr Pro Glu Pro Lys Leu Tyr Gly Gln Ile
            260                 265                 270
Phe Gly Gln Asn Val Ile Ile Gly Phe Val Leu Leu Ala Gly Met Leu
        275                 280                 285
Leu Gly Trp Leu
    290
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<210> 33
<211> 316
<212> PRT

<213> Synechocystis sp

<400> 33

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Met Val Thr Ser Thr Lys Ile His Arg Gln His Asp Ser Met Gly Ala
1               5                   10                  15
Val Cys Lys Ser Tyr Tyr Gln Leu Thr Lys Pro Arg Ile Ile Pro Leu
            20                  25                  30
Leu Leu Ile Thr Thr Ala Ala Ser Met Trp Ile Ala Ser Glu Gly Arg
        35                  40                  45
Val Asp Leu Pro Lys Leu Leu Ile Thr Leu Leu Gly Gly Thr Leu Ala
    50                  55                  60
Ala Ala Ser Ala Gln Thr Leu Asn Cys Ile Tyr Asp Gln Asp Ile Asp
65                  70                  75                  80
Tyr Glu Met Leu Arg Thr Arg Ala Arg Pro Ile Pro Ala Gly Lys Val
                85                  90                  95
Gln Pro Arg His Ala Leu Ile Phe Ala Leu Ala Leu Gly Val Leu Ser
            100                 105                 110
Phe Ala Leu Leu Ala Thr Phe Val Asn Val Leu Ser Gly Cys Leu Ala
        115                 120                 125
Leu Ser Gly Ile Val Phe Tyr Met Leu Val Tyr Thr His Trp Leu Lys
    130                 135                 140
Arg His Thr Ala Gln Asn Ile Val Ile Gly Gly Ala Ala Gly Ser Ile
145                 150                 155                 160
Pro Pro Leu Val Gly Trp Ala Ala Val Thr Gly Asp Leu Ser Trp Thr
                165                 170                 175
Pro Trp Val Leu Phe Ala Leu Ile Phe Leu Trp Thr Pro Pro His Phe
            180                 185                 190
Trp Ala Leu Ala Leu Met Ile Lys Asp Asp Tyr Ala Gln Val Asn Val
        195                 200                 205
Pro Met Leu Pro Val Ile Ala Gly Glu Glu Lys Thr Val Ser Gln Ile
    210                 215                 220
Trp Tyr Tyr Ser Leu Leu Val Val Pro Phe Ser Leu Leu Leu Val Tyr
225                 230                 235                 240
Pro Leu His Gln Leu Gly Ile Leu Tyr Leu Ala Ile Ala Ile Ile Leu
                245                 250                 255
Gly Gly Gln Phe Leu Val Lys Ala Trp Gln Leu Lys Gln Ala Pro Gly
            260                 265                 270
Asp Arg Asp Leu Ala Arg Gly Leu Phe Lys Phe Ser Ile Phe Tyr Leu
        275                 280                 285
Met Leu Leu Cys Leu Ala Met Val Ile Asp Ser Leu Pro Val Thr His
    290                 295                 300
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Gln Leu Val Ala Gln Met Gly Thr Leu Leu Leu Gly
305 310 315

<210> 34
<211> 324
<212> PRT
<213> Synechocystis sp

<400> 34
Met Ser Asp Thr Gln Asn Thr Gly Gln Asn Gln Ala Lys Ala Arg Gln
1 5 10 15
Leu Leu Gly Met Lys Gly Ala Ala Pro Gly Glu Ser Ser Ile Trp Lys
20 25 30
Ile Arg Leu Gln Leu Met Lys Pro Ile Thr Trp Ile Pro Leu Ile Trp
35 40 45
Gly Val Val Cys Gly Ala Ala Ser Ser Gly Gly Tyr Ile Trp Ser Val
50 55 60
Glu Asp Phe Leu Lys Ala Leu Thr Cys Met Leu Leu Ser Gly Pro Leu
65 70 75 80
Met Thr Gly Tyr Thr Gln Thr Leu Asn Asp Phe Tyr Asp Arg Asp Ile
85 90 95
Asp Ala Ile Asn Glu Pro Tyr Arg Pro Ile Pro Ser Gly Ala Ile Ser
100 105 110
Val Pro Gln Val Val Thr Gln Ile Leu Ile Leu Leu Val Ala Gly Ile
115 120 125
Gly Val Ala Tyr Gly Leu Asp Val Trp Ala Gln His Asp Phe Pro Ile
130 135 140
Met Met Val Leu Thr Leu Gly Gly Ala Phe Val Ala Tyr Ile Tyr Ser
145 150 155 160
Ala Pro Pro Leu Lys Leu Lys Gln Asn Gly Trp Leu Gly Asn Tyr Ala
165 170 175
Leu Gly Ala Ser Tyr Ile Ala Leu Pro Trp Trp Ala Gly His Ala Leu
180 185 190
Phe Gly Thr Leu Asn Pro Thr Ile Met Val Leu Thr Leu Ile Tyr Ser
195 200 205
Leu Ala Gly Leu Gly Ile Ala Val Val Asn Asp Phe Lys Ser Val Glu
210 215 220
Gly Asp Arg Gln Leu Gly Leu Lys Ser Leu Pro Val Met Phe Gly Ile
225 230 235 240
Gly Thr Ala Ala Trp Ile Cys Val Ile Met Ile Asp Val Phe Gln Ala
245 250 255

Gly Ile Ala Gly Tyr Leu Ile Tyr Val His Gln Gln Leu Tyr Ala Thr
260 265 270
Ile Val Leu Leu Leu Leu Ile Pro Gln Ile Thr Phe Gln Asp Met Tyr
275 280 285
Phe Leu Arg Asn Pro Leu Glu Asn Asp Val Lys Tyr Gln Ala Ser Ala
290 295 300
Gln Pro Phe Leu Val Phe Gly Met Leu Ala Thr Gly Leu Ala Leu Gly
305 310 315 320
His Ala Gly Ile

<210> 35
<211> 307
<212> PRT
<213> Synechocystis sp

<400> 35
Met Thr Glu Ser Ser Pro Leu Ala Pro Ser Thr Ala Pro Ala Thr Arg
1 5 10 15
Lys Leu Trp Leu Ala Ala Ile Lys Pro Pro Met Tyr Thr Val Ala Val
20 25 30
Val Pro Ile Thr Val Gly Ser Ala Val Ala Tyr Gly Leu Thr Gly Gln
35 40 45
Trp His Gly Asp Val Phe Thr Ile Phe Leu Leu Ser Ala Ile Ala Ile
50 55 60
Ile Ala Trp Ile Asn Leu Ser Asn Asp Val Phe Asp Ser Asp Thr Gly
65 70 75 80
Ile Asp Val Arg Lys Ala His Ser Val Val Asn Leu Thr Gly Asn Arg
85 90 95
Asn Leu Val Phe Leu Ile Ser Asn Phe Phe Leu Leu Ala Gly Val Leu
100 105 110
Gly Leu Met Ser Met Ser Trp Arg Ala Gln Asp Trp Thr Val Leu Glu
115 120 125
Leu Ile Gly Val Ala Ile Phe Leu Gly Tyr Thr Tyr Gln Gly Pro Pro
130 135 140
Phe Arg Leu Gly Tyr Leu Gly Leu Gly Glu Leu Ile Cys Leu Ile Thr
145 150 155 160
Phe Gly Pro Leu Ala Ile Ala Ala Ala Tyr Tyr Ser Gln Ser Gln Ser
165 170 175
Phe Ser Trp Asn Leu Leu Thr Pro Ser Val Phe Val Gly Ile Ser Thr
180 185 190

Ala Ile Ile Leu Phe Cys Ser His Phe His Gln Val Glu Asp Asp Leu
195 200 205
Ala Ala Gly Lys Lys Ser Pro Ile Val Arg Leu Gly Thr Lys Leu Gly
210 215 220
Ser Gln Val Leu Thr Leu Ser Val Val Ser Leu Tyr Leu Ile Thr Ala
225 230 235 240
Ile Gly Val Leu Cys His Gln Ala Pro Trp Gln Thr Leu Leu Ile Ile
245 250 255
Ala Ser Leu Pro Trp Ala Val Gln Leu Ile Arg His Val Gly Gln Tyr
260 265 270
His Asp Gln Pro Glu Gln Val Ser Asn Cys Lys Phe Ile Ala Val Asn
275 280 285
Leu His Phe Phe Ser Gly Met Leu Met Ala Ala Gly Tyr Gly Trp Ala
290 295 300
Gly Leu Gly
305

<210> 36
<211> 927
<212> DNA
<213> Synechocystis sp

<400> 36
atggcaacta tccaagcttt ttggcgcttc tcccgccccc ataccatcat tggtacaact 60
ctgagcgtct gggctgtgta tctgttaact attctcgggg atggaaactc agttaactcc 120
cctgcttccc tggatttagt gttcggcgct tggctggcct gcctgttggg taatgtgtac 180
attgtcggcc tcaaccaatt gtgggatgtg gacattgacc gcatcaataa gccgaatttg 240
cccctagcta acggagattt ttctatcgcc cagggccgtt ggattgtggg actttgtggc 300
gttgcttcct tggcgatcgc ctggggatta gggctatggc tggggctaac ggtgggcatt 360
agtttgatta ttggcacggc ctattcggtg ccgccagtga ggttaaagcg cttttccctg 420
ctggcggccc tgtgtattct gacggtgcgg ggaattgtgg ttaacttggg cttattttta 480
ttttttagaa ttggtttagg ttatcccccc actttaataa cccccatctg ggttttgact 540
ttatttatct tagttttcac cgtggcgatc gccattttta aagatgtgcc agatatggaa 600
ggcgatcggc aatttaagat tcaaacttta actttgcaaa tcggcaaaca aaacgttttt 660
cggggaacct taattttact cactggttgt tatttagcca tggcaatctg ggcttatggg 720
gcggctatgc ctttaaatac tgctttcttg attgtttccc atttgtgctt attagcctta 780
ctctggtggc ggagtcgaga tgtacactta gaaagcaaaa ccgaaattgc tagtttttat 840
cagtttattt ggaagctatt tttcttagag tacttgctgt atcccttggc tctgtggtta 900
cctaattttt ctaatactat tttttag 927

<210> 37

<211> 308

<212> PRT

<213> Synechocystis sp

<400> 37

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Met Ala Thr Ile Gln Ala Phe Trp Arg Phe Ser Arg Pro His Thr Ile
 1               5                  10                  15
Ile Gly Thr Thr Leu Ser Val Trp Ala Val Tyr Leu Leu Thr Ile Leu
            20                  25                  30
Gly Asp Gly Asn Ser Val Asn Ser Pro Ala Ser Leu Asp Leu Val Phe
        35                  40                  45
Gly Ala Trp Leu Ala Cys Leu Leu Gly Asn Val Tyr Ile Val Gly Leu
    50                  55                  60
Asn Gln Leu Trp Asp Val Asp Ile Asp Arg Ile Asn Lys Pro Asn Leu
65                  70                  75                  80
Pro Leu Ala Asn Gly Asp Phe Ser Ile Ala Gln Gly Arg Trp Ile Val
                85                  90                  95
Gly Leu Cys Gly Val Ala Ser Leu Ala Ile Ala Trp Gly Leu Gly Leu
            100                 105                 110
Trp Leu Gly Leu Thr Val Gly Ile Ser Leu Ile Ile Gly Thr Ala Tyr
        115                 120                 125
Ser Val Pro Pro Val Arg Leu Lys Arg Phe Ser Leu Leu Ala Ala Leu
    130                 135                 140
Cys Ile Leu Thr Val Arg Gly Ile Val Val Asn Leu Gly Leu Phe Leu
145                 150                 155                 160
Phe Phe Arg Ile Gly Leu Gly Tyr Pro Pro Thr Leu Ile Thr Pro Ile
                165                 170                 175
Trp Val Leu Thr Leu Phe Ile Leu Val Phe Thr Val Ala Ile Ala Ile
            180                 185                 190
Phe Lys Asp Val Pro Asp Met Glu Gly Asp Arg Gln Phe Lys Ile Gln
        195                 200                 205
Thr Leu Thr Leu Gln Ile Gly Lys Gln Asn Val Phe Arg Gly Thr Leu
    210                 215                 220
Ile Leu Leu Thr Gly Cys Tyr Leu Ala Met Ala Ile Trp Gly Leu Trp
225                 230                 235                 240
Ala Ala Met Pro Leu Asn Thr Ala Phe Leu Ile Val Ser His Leu Cys
                245                 250                 255
Leu Leu Ala Leu Leu Trp Trp Arg Ser Arg Asp Val His Leu Glu Ser
            260                 265                 270
Lys Thr Glu Ile Ala Ser Phe Tyr Gln Phe Ile Trp Lys Leu Phe Phe
        275                 280                 285
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Leu Glu Tyr Leu Leu Tyr Pro Leu Ala Leu Trp Leu Pro Asn Phe Ser
290 295 300

Asn Thr Ile Phe
305

<210> 38
<211> 1092
<212> DNA
<213> Synechocystis sp

<400> 38
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atgaaatttc cgccccacag tggttaccat tggcaaggtc aatcaccttt ctttgaaggt      60
tggtacgtgc gcctgctttt gccccaatcc ggggaaagtt ttgcttttat gtactccatc     120
gaaaatcctg ctagcgatca tcattacggc ggcggtgctg tgcaaatttt agggccggct     180
acgaaaaaac aagaaaatca ggaagaccaa cttgtttggc ggacatttcc ctcggtaaaa     240
aaattttggg ccagtcctcg ccagtttgcc ctagggcatt ggggaaaatg tagggataac     300
aggcaggcga aaccccctact ctccgaagaa ttttttgcca cggtcaagga aggttatcaa     360
atccatcaaa atcagcacca aggacaaatc attcatggcg atcgccattg tcgttggcag     420
ttcaccgtag aaccggaagt aacttggggg agtcctaacc gatttcctcg ggctacagcg     480
ggttggcttt cctttttacc cttgtttgat cccggttggc aaattctttt agcccaaggt     540
agagcgcacg gctggctgaa atggcagagg gaacagtatg aatttgacca cgccctagtt     600
tatgccgaaa aaaattgggg tcactccttt ccctcccgct ggttttggct ccaagcaaat     660
tattttcctg accatccagg actgagcgtc actgccgctg gcggggaacg gattgttctt     720
ggtcgccccg aagaggtagc tttaattggc ttacatcacc aaggtaattt ttacgaattt     780
ggcccgggcc atggcacagt cacttggcaa gtagctccct ggggccgttg gcaattaaaa     840
gccagcaatg ataggtattg ggtcaagttg tccggaaaaa cagataaaaa aggcagttta     900
gtccacactc ccaccgccca gggcttacaa ctcaactgcc gagataccac taggggctat     960
ttgtatttgc aattgggatc tgtgggtcac ggcctgatag tgcaagggga aacggacacc    1020
gcggggctag aagttggagg tgattggggt ttaacagagg aaaatttgag caaaaaaaca    1080
gtgccattct ga                                                        1092
```

<210> 39
<211> 363
<212> PRT
<213> Synechocystis sp

<400> 39
Met Lys Phe Pro Pro His Ser Gly Tyr His Trp Gln Gly Gln Ser Pro
1 5 10 15
Phe Phe Glu Gly Trp Tyr Val Arg Leu Leu Leu Pro Gln Ser Gly Glu
20 25 30

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Ser Phe Ala Phe Met Tyr Ser Ile Glu Asn Pro Ala Ser Asp His His
        35                  40                  45
Tyr Gly Gly Gly Ala Val Gln Ile Leu Gly Pro Ala Thr Lys Lys Gln
    50                  55                  60
Glu Asn Gln Glu Asp Gln Leu Val Trp Arg Thr Phe Pro Ser Val Lys
65                  70                  75                  80
Lys Phe Trp Ala Ser Pro Arg Gln Phe Ala Leu Gly His Trp Gly Lys
                85                  90                  95
Cys Arg Asp Asn Arg Gln Ala Lys Pro Leu Leu Ser Glu Glu Phe Phe
            100                 105                 110
Ala Thr Val Lys Glu Gly Tyr Gln Ile His Gln Asn Gln His Gln Gly
        115                 120                 125
Gln Ile Ile His Gly Asp Arg His Cys Arg Trp Gln Phe Thr Val Glu
    130                 135                 140
Pro Glu Val Thr Trp Gly Ser Pro Asn Arg Phe Pro Arg Ala Thr Ala
145                 150                 155                 160
Gly Trp Leu Ser Phe Leu Pro Leu Phe Asp Pro Gly Trp Gln Ile Leu
                165                 170                 175
Leu Ala Gln Gly Arg Ala His Gly Trp Leu Lys Trp Gln Arg Glu Gln
            180                 185                 190
Tyr Glu Phe Asp His Ala Leu Val Tyr Ala Glu Lys Asn Trp Gly His
        195                 200                 205
Ser Phe Pro Ser Arg Trp Phe Trp Leu Gln Ala Asn Tyr Phe Pro Asp
    210                 215                 220
His Pro Gly Leu Ser Val Thr Ala Ala Gly Gly Glu Arg Ile Val Leu
225                 230                 235                 240
Gly Arg Pro Glu Glu Val Ala Leu Ile Gly Leu His His Gln Gly Asn
                245                 250                 255
Phe Tyr Glu Phe Gly Pro Gly His Gly Thr Val Thr Trp Gln Val Ala
            260                 265                 270
Pro Trp Gly Arg Trp Gln Leu Lys Ala Ser Asn Asp Arg Tyr Trp Val
        275                 280                 285
Lys Leu Ser Gly Lys Thr Asp Lys Lys Gly Ser Leu Val His Thr Pro
    290                 295                 300
Thr Ala Gln Gly Leu Gln Leu Asn Cys Arg Asp Thr Thr Arg Gly Tyr
305                 310                 315                 320
Leu Tyr Leu Gln Leu Gly Ser Val Gly His Gly Leu Ile Val Gln Gly
                325                 330                 335
Glu Thr Asp Thr Ala Gly Leu Glu Val Gly Gly Asp Trp Gly Leu Thr
            340                 345                 350
Glu Glu Asn Leu Ser Lys Lys Thr Val Pro Phe
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355 360

<210> 40
<211> 56
<212> DNA
<213> Artifical Sequence

<400> 40
cgcgatttaa atggcgcgcc ctgcaggcgg ccgcctgcag ggcgcgccat ttaaat 56

<210> 41
<211> 32
<212> DNA
<213> Artifical Sequence

<400> 41
tcgaggatcc gcggccgcaa gcttcctgca gg 32

<210> 42
<211> 32
<212> DNA
<213> Artifical Sequence

<400> 42
tcgacctgca ggaagcttgc ggccgcggat cc 32

<210> 43
<211> 32
<212> DNA
<213> Artifical Sequence

<400> 43
tcgacctgca ggaagcttgc ggccgcggat cc 32

<210> 44
<211> 32
<212> DNA
<213> Artifical Sequence

<400> 44
tcgaggatcc gcggccgcaa gcttcctgca gg 32

<210> 45
<211> 36
<212> DNA
<213> Artifical Sequence

<400> 45
tcgaggatcc gcggccgcaa gcttcctgca ggagct 36

<210> 46
<211> 28
<212> DNA
<213> Artifical Sequence

<400> 46
cctgcaggaa gcttgcggcc gcggatcc 28

<210> 47
<211> 36
<212> DNA
<213> Artifical Sequence

<400> 47
tcgacctgca ggaagcttgc ggccgcggat ccagct 36

<210> 48
<211> 28
<212> DNA
<213> Artifical Sequence

<400> 48
ggatccgcgg ccgcaagctt cctgcagg 28

<210> 49
<211> 39
<212> DNA
<213> Artifical Sequence

<400> 49
gatcacctgc aggaagcttg cggccgcgga tccaatgca 39

<210> 50
<211> 31
<212> DNA
<213> Artifical Sequence

<400> 50
ttggatccgc ggccgcaagc ttcctgcagg t 31

<210> 51
<211> 41
<212> DNA
<213> Artifical Sequence

<400> 51
ggatccgcgg ccgcacaatg gagtctctgc tctctagttc t 41

<210> 52
<211> 38
<212> DNA
<213> Artifical Sequence

<400> 52
ggatcctgca ggtcacttca aaaaaggtaa cagcaagt 38

<210> 53
<211> 45
<212> DNA
<213> Artifical Sequence

<400> 53
ggatccgcgg ccgcacaatg gcgtttttttg ggctctcccg tgttt 45

<210> 54
<211> 40
<212> DNA
<213> Artifical Sequence

<400> 54
ggatcctgca ggttattgaa aacttcttcc aagtacaact 40

<210> 55

<211> 38
<212> DNA
<213> Artifical Sequence

<400> 55
ggatccgcgg ccgcacaatg tggcgaagat ctgttgtt 38

<210> 56
<211> 37
<212> DNA
<213> Artifical Sequence

<400> 56
ggatcctgca ggtcatggag agtagaagga aggagct 37

<210> 57
<211> 50
<212> DNA
<213> Artifical Sequence

<400> 57
ggatccgcgg ccgcacaatg gtacttgccg aggttccaaa gcttgcctct 50

<210> 58
<211> 38
<212> DNA
<213> Artifical Sequence

<400> 58
ggatcctgca ggtcacttgt ttctggtgat gactctat 38

<210> 59
<211> 38
<212> DNA
<213> Artifical Sequence

<400> 59
ggatccgcgg ccgcacaatg acttcgattc tcaacact 38

<210> 60
<211> 36

<212> DNA
<213> Artifical Sequence

<400> 60
ggatcctgca ggtcagtgtt gcgatgctaa tgccgt 36

<210> 61
<211> 22
<212> DNA
<213> Artifical Sequence

<400> 61
taatgtgtac attgtcggcc tc 22

<210> 62
<211> 60
<212> DNA
<213> Artifical Sequence

<400> 62
gcaatgtaac atcagagatt ttgagacaca acgtggcttt ccacaattcc ccgcaccgtc 60

<210> 63
<211> 22
<212> DNA
<213> Artifical Sequence

<400> 63
aggctaataa gcacaaatgg ga 22

<210> 64
<211> 63
<212> DNA
<213> Artifical Sequence

<400> 64
ggtatgagtc agcaacacct tcttcacgag gcagacctca gcggaattgg tttaggttat 60
ccc 63

<210> 65
<211> 26

<212> DNA
<213> Artifical Sequence

<400> 65
ggatccatgg ttgcccaaac cccatc 26

<210> 66
<211> 61
<212> DNA
<213> Artifical Sequence

<400> 66
gcaatgtaac atcagagatt ttgagacaca acgtggcttt gggtaagcaa caatgaccgg 60
c 61

<210> 67
<211> 25
<212> DNA
<213> Artifical Sequence

<400> 67
gaattctcaa agccagccca gtaac 25

<210> 68
<211> 63
<212> DNA
<213> Artifical Sequence

<400> 68
ggtatgagtc agcaacacct tcttcacgag gcagacctca gcgggtgcga aaagggtttt 60
ccc 63

<210> 69
<211> 23
<212> DNA
<213> Artifical Sequence

<400> 69
ccagtggttt aggctgtgtg gtc 23

<210> 70

<211> 21
<212> DNA
<213> Artifical Sequence

<400> 70
ctgagttgga tgtattggat c 21

<210> 71
<211> 28
<212> DNA
<213> Artifical Sequence

<400> 71
ggatccatgg ttacttcgac aaaaatcc 28

<210> 72
<211> 60
<212> DNA
<213> Artifical Sequence

<400> 72
gcaatgtaac atcagagatt ttgagacaca acgtggcttt gctaggcaac cgcttagtac 60

<210> 73
<211> 28
<212> DNA
<213> Artifical Sequence

<400> 73
gaattcttaa cccaacagta aagttccc 28

<210> 74
<211> 63
<212> DNA
<213> Artifical Sequence

<400> 74
ggtatgagtc agcaacacct tcttcacgag gcagacctca gcgccggcat tgtcttttac 60
atg 63

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<210> 95
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<212> DNA
<213> soy

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<210> 96
<211>
<212> DNA
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<210> 97

<211>

<212> PRT

<213> soy

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<210> 98

<211>

<212> PRT

<213> soy

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<210> 99

<211>

<212> DNA

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<210> 100

<211>

<212> DNA

<213> wheat

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<210> 101

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<212> DNA

<213> leek

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<210> 102

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<212> DNA

<213> leek

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<212> DNA

<213> canola

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<210> 104
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<212> DNA
<213> corn

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<210> 105
<211>
<212> DNA
<213> corn

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<210> 106

<211>

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<213> corn

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<210> 107

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<212> DNA

<213> cotton

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<210> 108

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<213> tomato

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